doi:10.7289/V5H70CTG

Cetacean Monitoring in the Mariana Islands Range Complex, 2015[1]

Marie C. Hill[1,2], Erin M. Oleson[2], Simone Baumann-Pickering[3], Amy M. VanCise[3,4], Allan D. Ligon[6], Andrea R. Bendlin[5], Adam C. Ü[5,7], Jennifer S. Trickey[3], and Amanda L. Bradford[2]

[1] Joint Institute for Marine and Atmospheric Research
Research Corporation of the University of Hawai'i
1000 Pope Road, Honolulu, Hawai'i 96822, U.S.A.

[2]NOAA Fisheries
Pacific Islands Fisheries Science Center
1845 Wasp Blvd. Building 176, Honolulu, Hawai'i 96818, U.S.A.

[3]University of California San Diego
Scripps Institution of Oceanography
9500 Gilman Dr. #0203, La Jolla, CA 92093

[4]NOAA Fisheries
Southwest Fisheries Science Center
8901 La Jolla Shores Drive, La Jolla CA 92037

[5]Ocean Associates contractor for
Pacific Islands Fisheries Science Center
1845 Wasp Blvd., Building 176, Honolulu, Hawai'i 96818, U.S.A.

[6] 3341 N 27th Ave, Unit 27, Bozeman, Montana 59718, U.S.A.

[7]PO Box 1329, Maple Falls, Washington 98266, U.S.A.

Suggested citation:

Hill M.C., E.M. Oleson, S. Baumann-Pickering, A.M. VanCise, A.D. Ligon, A.R. Bendlin, A.C. Ü, J.S. Trickey, and A.L. Bradford. 2016. Cetacean Monitoring in the Mariana Islands Range Complex, 2015. Prepared for the U.S. Pacific Fleet Environmental Readiness Office. PIFSC Data Report DR-16-01. 36 pp. + Appendix

[1]NOAA Pacific Islands Fisheries Science Center
PIFSC Data Report DR-16-001
Issued 5 February 2016

Mission

The Pacific Islands Fisheries Science Center's (PIFSC) Cetacean Research Program (CRP) has been conducting visual surveys and long-term acoustic monitoring for cetaceans in the waters surrounding Guam and the Commonwealth of the Northern Mariana Islands (CNMI) as part of an ongoing effort to develop a record of cetacean occurrence in the region. Visual surveys have been conducted aboard small boats (7.6–12.2 m)since 2010 off the southernmost islands of the Mariana Archipelago (Guam, Rota, Saipan, Tinian, and Aguijan). These surveys include the collection of photographs for individual identification, tissue samples for genetic analysis of population structure, and the deployment of satellite tags for assessment of individual movements throughout the broader region. These surveys are conducted in partnership with the Commander, U.S. Pacific Fleet Environmental Readiness Division. PIFSC has been collecting long-term passive acoustic monitoring at two sites near Saipan and Tinian since 2010. Analyses of these data sets have included detection of a variety of cetacean species, primarily baleen whales, sperm whales, and beaked whales. Data sets from the various visual and acoustic efforts are used to evaluate the seasonal occurrence and distribution, stock structure, and movements of cetaceans within the study area. This report includes a summary of the most recent visual surveys that were conducted in the winter (February–March) and summer (August–September) of 2015, the movements of a false killer whale (*Pseudorca crassidens*) that were satellite tagged during the PIFSC Mariana Archipelago Cetacean Survey in May–June, 2015, the results of nuclear and mitochondrial analysis of short-finned pilot whale samples collected during small-boat surveys of the southernmost Mariana islands (2011–2014), and analysis of beaked whale occurrence long-term passive acoustic data set for 2014–15. Analyses of acoustic data for baleen whales are still underway and will be reported at a later time.

The observer team conducted the winter visual surveys off Saipan and Tinian to specifically look for humpback whales (*Megaptera novaeangliae*), which we know occur there seasonally based on sightings during a 2007 shipboard survey (Fulling et al. 2011), from acoustic recordings (Hill et al. 2015), from anecdotal reports of fishermen and recreational dive operators, and from a boat-strike incident in 2014 (unpublished). In addition to small boat surveys, the observer team conducted four shore-based surveys to evaluate the feasibility of sighting whales from shore on days when conditions were too rough to conduct small boat surveys.

Our summer visual surveys were a continuation of prior year surveys and were broader in scope; e.g., not focused on a specific species but an assessment of the occurrence of multiple species. Unlike previous years, the observer team did not survey around Saipan, Tinian, and

Aguijan. The week prior to our arrival, the devastation of Saipan by Typhoon Soudelor precluded our ability to conduct surveys there.

Methods

Field Methods

Winter Visual Survey

The PIFSC CRP conducted visual surveys for humpback whales from a shore station and from a small boat during February–March 2015. The observer team conducted shore-based observations from an elevated station that overlooked the central-west side of Saipan (Fig. 1). The location was chosen based on accounts of humpback whale sightings by local fisherman and dive operators. Observers scanned the waters beyond the outer reef using 10 × 50 binoculars with reticles and compass mounted on tripods. Approximate locations of humpback whale blows were recorded using 10 × 50 binoculars with reticles and bearings. The observer team also conducted small-boat surveys aboard the *Sea Hunter*. Rough sea conditions limited surveys primarily to the west side of Saipan, and the observer team focused on areas where humpback whale blows were seen from the shore observation station as well as estimated locations from local visual accounts of fisherman and dive boat operators. The team attempted to approach each individual whale or group for photo-identification and biopsy sampling and used the same photo-identification and biopsy protocols as those described by Hill et al. (2014).

The observer team recorded the occurrences and locations of turtles but did not collect photos or biological samples.

Summer Visual Survey

The PIFSC CRP conducted visual surveys for cetaceans from small vessels off Guam and Rota in summer 2015. During August–September, the observer team conducted surveys aboard 2 different vessels (*Ten27* and *Proline*) while off Guam and used a single vessel (*Asakaze*) while working off Rota, as well as the transit survey from Rota to Guam. The CRP designed the visual survey effort to cover representative habitat within the study area and the observer team spread out the vessel tracks from day to day to ensure broad survey coverage over a wide range of depths. Weather and sea conditions also dictated the direction and scope of the survey effort. The survey vessels traveled at a speed of 15–26 km/h, depending on the size of the vessel and sea conditions. Captains allowed the observer team to operate the vessel when approaching cetaceans for photo-identification, biopsy, and satellite tagging. Four to 5 observers scanned for marine mammals with unaided eye, collectively searching 360 degrees around the vessel.

The observer team approached all cetacean groups for species confirmation, group size estimates, and photo-identification and during encounters with certain species conducted biopsy sampling and satellite tagging operations. The team used the same photo-identification and biopsy protocols as those described by Hill et al. (2014) and the same satellite tagging protocols as those described by Hill et al. (2014, 2015).

The observer team recorded the occurrences and locations of turtles but did not collect photos or biological samples.

Acoustic Analysis from Long-term Data Sets

PIFSC maintains long-term acoustic data sets collected near Saipan and Tinian in the southern Mariana Archipelago. High-frequency Acoustic Recording Packages (HARPs) were used to record underwater sounds from 10 Hz to 100 kHz with 16-bit quantization. The HARP sensor and mooring package are described in Wiggins and Hildebrand (2007). Each HARP is calibrated in the laboratory to provide a quantitative analysis of the received sound field. Representative data loggers and hydrophones have also been calibrated at the Navy's Transducer Evaluation Center (TRANSDEC) facility to verify the laboratory calibrations. Deployment and recording details for acoustic data sets analyzed as part of this effort are provided in Table 1.

The full bandwidth HARP data set was used for detection and classification of beaked whales. The detection and classification process was the same as that used in previous analyses of the Saipan and Tinian HARP data sets (Oleson et al. 2015, Hill et al. 2015) and will be summarized briefly here. An automated multi-step beaked whale detector (Baumann-Pickering et al. 2013) was run on the data set to detect periods of beaked whale calling. Echolocation signals were initially identified with a click detector (Soldevilla et al. 2008, Roch et al. 2011), and then individual click detections were band-pass filtered between 5 kHz and 95 kHz. Spectra of each detected signal were used to measure peak frequency, center frequency, and bandwidth. Duration was derived based on the detector output and sweep rate was computed with spectrograms over 1.2 ms of data centered on the signal. A line of best fit through the sweep was calculated, resulting in a sweep rate. All detected echolocation signals, independent of distance and orientation of the recorded animal with respect to the recorder, were included in the analysis.

Beaked whale echolocation click encounters were classified to species using computer assisted manual decisions by trained analysts, which labeled the automatically detected segments to species level and rejected false detections (method in Baumann-Pickering et al. 2013). For each acoustic encounter histograms of peak frequency and IPI, their medians, and those of peak-to-peak received level, center frequency, and duration were displayed. Mean spectra of all pulses and mean noise preceding each FM pulse was plotted against an overlay of spectral templates

from all currently known FM pulse types (see Baumann-Pickering et al. (2013) for descriptions). The overlay of the mean spectra of the encounter with the spectral templates allowed for comparison of all spectral features, with special emphasis on smaller spectral peaks at frequencies below the main energy content and the slope at which the main energy content rose. A final judgment about the signal type was based on comparison to the templates and labeled to species or unknown signal-type level by the analyst. This detection and classification system does not identify clicks produced by Longman's beaked whales because this species produces only very few FM pulse type signals and considerably more delphinid-like echolocation clicks (Rankin et al. 2011). Those signals are not currently distinguishable from dolphin echolocation clicks.

Data Processing and Analyses

Visual Surveys and Encounters

For processing and analyzing the visual survey and encounter data, the CRP used the same methods and bathymetry data as those described in Hill et al. (2014) and augmented the previously used bathymetry data in offshore areas with the Global Multi-Resolution Topography (GMRT)[2] custom bathymetric grid encompassing the Commonwealth of the Northern Mariana Islands (CNMI) and Guam Exclusive Economic Zone (EEZ), as well as areas outside of the EEZ boundary in the Western Pacific Ocean and Philippine Sea. The GMRT is a multi-resolution gridded global Digital Elevation Model (DEM) that includes cleaned, processed ship-based multibeam sonar data at their full spatial resolution (Ryan et al. 2009). Where multibeam sonar data are not available, the GMRT uses gridded seafloor depth data (30 arc-second resolution) from the General Bathymetric Chart of the Oceans (Weatherall et al. 2015).

Satellite Telemetry

To process and analyze the satellite tag location data, the CRP used the same methods as those described in Hill et al. (2014, 2015). Data included in these analyses derived from satellite tags deployed during the summer small-boat effort, as well as the PIFSC ship-based Mariana Archipelago Cetacean Survey in May–June, 2015.

Photo-Identification

Photo processing and analysis was continued to add to existing individual photo-identification catalogs and the CRP used the same protocols as those described in Hill et al. (2014). To assess the feasibility and value of creating a photo-identification catalog of pantropical spotted dolphins (*Stenella attenuata*) for the purpose of mark-recapture analysis and population abundance estimation, photo analysts evaluated the proportion of marked

[2]Marine Geoscience Data System http://www.marine-geo.org/portals/gmrt

dolphins within groups encountered off Guam 2010-2014 (n = 12). They looked for very distinctive individuals to assess the re-sight potential in the data set. If individuals were not well marked or ever re-sighted, mark-recapture methods would not be appropriate for estimating abundance, such that creation of complete photo-identification catalogs for this species would have little value.

Short-finned Pilot Whale Genetics

Van Cise et al. (Appendix I) conducted sequencing of nuclear SNP data from biopsy samples collected from short-finned pilot whales in the Marianas, Hawaii, and the eastern tropical Pacific for the purposes of the degree of relatedness among pilot whales in these regions. They sequenced 78 targeted nuclear loci for SNP analysis of short-finned pilot whale DNA using a custom capture enrichment array designed at SWFSC, followed by highly-parallel sequencing (Hancock-Hanser et al. 2013). Candidate SNPs for each locus were manually selected; SNPs with coverage at less than 35% of samples were removed from the study, then samples with coverage at less than 50% of the selected SNPs were removed from the study. Loci with multiple SNPs were phased to generate a single genotype per locus for analysis of population structure at both regional and local stratifications, including Fst, ΦST and Bayesian analysis in STRUCTURE 2.3.3. Additionally, relatedness analysis were conducted between photographically identified Hawaiian social groups using a set of high heterozygosity SNPs, analyzed using the related package for R (Pew et al. 2014), which implements the software program COANCESTRY (Wang 2011). Detailed methods can be found in Appendix I.

Results

Visual Surveys and Encounters

Winter

On 24, 25, 27 February and 5 March, the PIFSC CRP conducted shore-based observations to look for humpback whales from an elevated station (Fig. 1). On 24 February the observer team spotted a breaching whale from the observation station while assessing the setup of the station. The team could not confirm that it was a humpback whale but the location was consistent with those of previous anecdotal reports. The breaching whale was located approximately 6-7 km offshore, on top of Chalan Kanoa (CK) Reef (a.k.a. Double Reef or 6-mile Reef). The team confirmed the presence of humpback whales around the observed location during subsequent small boat surveys, and also observed humpback whale blows from the observation station on 25, 27 February and 5 March.

Between 26 February and 8 March, the observer team conducted small-boat visual surveys within the waters off the west sides of Saipan and Tinian and surveyed 522 km of trackline (Table 2, Fig. 1). Beaufort sea states along most of the on-effort trackline (98%, 513

km) ranged 4-6 and dominant swell heights were 4-8 ft (82%, 426 km) (Fig. 2). After initially finding whales during multiple days on CK Reef, the team continued to focus most of the survey efforts there and spent more than half (58%, 24.7 hr) of the on-effort time surveying over water depths of 0-100 m (Fig. 3).

The observer team had 29 encounters with 3 cetacean species including humpback whales, bottlenose dolphins (*Tursiops truncatus*), and pygmy killer whales (*Feresa attenuata*) (Table 3, Fig. 1). All but 2 of the encounters were on CK Reef (Table 3, Fig. 1). Photographs confirmed the presence of 4 humpback whale mom/calf pairs and 4 other humpback whale individuals. The team collected fluke photos from two individuals and used body and dorsal hump characteristics to distinguish the others. Additional whales may have been present but could not be confirmed with photographs. The team encountered 2 of the 4 mom/calf pairs on multiple days and collected biopsy samples from 3 of the moms. During one encounter on CK reef, two adult humpback whales socialized together along with 6 pygmy killer whales, and the team was able to collect a biopsy sample from one of the humpbacks.

The survey team observed a total of 18 turtles (Table 5) and 2 single juvenile whale sharks (*Rhincodon typus*).

Summer

The PIFSC CRP conducted small-boat visual surveys within the waters surrounding Guam on 13–26 August and 4–8 September and Rota on 28 August–2 September. In addition, the observer team visually surveyed during the transit from Rota to Guam on 3 September. The team surveyed a total of 2,092 km of trackline during which more than half was in Beaufort sea states of 0–3 (66%, 1387 km) and swell heights of 0–4 ft (62%, 1296 km) (Table 4, Figs. 4–5). A little more than 10% (12.6 hr) of the total time on effort was spent inside of the 100-m depth contour (Fig. 6). Effort was distributed fairly evenly over 10–1300-m depth bins with slightly more effort over the 600–900 m range and was reduced gradually over depths of 1300–2700 m (Fig. 6).

The observer team encountered 27 cetacean groups during the small-boat surveys off Guam and Rota, resulting in the collection of more than 8,000 photos and 20 biopsy samples (Table 4, Fig. 4). The 25 encountered cetacean groups, identified to species, included bottlenose dolphins, Blainville's beaked whales (*Mesoplodon densirostris*), Bryde's whales (*Balaenoptera edeni*), false killer whales (*Pseudorca crassidens*), pantropical spotted dolphins (*Stenella attenuata*), pygmy killer whales, and spinner dolphins (*Stenella longirostris*). The encounters included one mixed group of pantropical spotted dolphins and bottlenose dolphins, and one sighting of a single whale that the observer team identified as sei/Bryde's because they were not able to confirm the presence of the distinctive rostral ridges in the field or in

photographs. A biopsy sample, collected from this sei/Bryde's whale, will eventually provide species confirmation. During the surveys off Rota, the team encountered one unidentified whale that they observed only as a blow in the distance, but did not relocate.

Pantropical spotted dolphins were the most frequently sighted species (n = 10) during the summer surveys and most encounters were off Guam (Tables 3, 5; Fig. 4). During a mixed species encounter with bottlenose dolphins off Rota, the spotted dolphins swam with and were chased by the bottlenose dolphins. Group sizes ranged 13–121 individuals and Young of the Year (YOY) or neonates were present during 6 encounters (Table 4). The observer team collected 3 biopsy samples from a group encountered off the southwest side Guam in a location where they had not seen spotted dolphins before (Table 4, Fig. 4).

Spinner dolphins were the second most frequently sighted species (n = 6) (Table 4, Fig. 4). Group sizes ranged 1–101 individuals and YOY or neonates were present during 50% of the encounters. The observer team had one encounter off Guam, just outside of Tumon Bay, with over 100 individuals and recognized some individuals during the encounter. Preliminary matching to the photo-identification catalog found that some of these individuals had been photographed off the north and northeast sides of Guam, as well as on Rota Bank in previous years.

The observer team had 4 bottlenose dolphin encounters, all of which were off Rota (Table 4, Fig. 4). Group sizes ranged 3–27 individuals. The first encounter with a group of 27 individuals including a neonate occurred on 30 August near Ice Box Reef during which the team collected 9 biopsy samples (Fig. 4). A preliminary scan of the photos resulted in no matches to the photo-identification catalog. The other 3 encounters occurred on 1 and 2 September and were with individuals that the observer team recognized from the photo-identification catalog. The second and fourth encounters were both close to shore (300 m) but were off opposite sides of the island (Table 4, Fig. 4). Several of the individuals from the previous day were present on 2 September. The observer team deployed a satellite tag on one individual and collected a biopsy sample from a different individual. Earlier in the day on 2 September the team encountered 3 bottlenose dolphins that were interacting with a group of pantropical spotted dolphins 8.6 km offshore. The bottlenose dolphins swam with spotted dolphins and chased them. The observer team collected 1 biopsy sample from a bottlenose dolphin (Table 4). All 3 bottlenose dolphins were present during the nearshore encounter later in the day.

The observer team encountered 3 Bryde's whales with species identification confirmed by photos (3 ridges clearly visible on the rostrum) and a fourth potential Bryde's whale (sei/Bryde's) for which the team was unable to get good rostrum photos. All were different individual whales and were difficult to approach. While working off Rota, the team encountered the first of the 3 Bryde's whales, as well as the sei/Bryde's and collected biopsy

samples from both (Table 4, Fig. 4). During the transit from Rota to Guam, the team encountered a lunge-feeding Bryde's whale at the north edge of Rota Bank but was unable to approach it to collect a biopsy sample. The last Bryde's whale encounter occurred off Guam, near the Ledge Buoy. It was a juvenile whale, much smaller than the other 3 whales. The observer team collected both species identifying photos and a biopsy sample.

On 14 August, the observer team encountered a group of 11 pygmy killer whales off the southwest side of Guam and recognized 9 of these individuals from encounters in previous years (Hill et al. 2014, 2015) (Table 4, Fig. 4). The team collected 1 biopsy sample from an individual that was biopsied in 2013.

On 28 August 2015, the observer team encountered a single group of Blainville's beaked whales off Rota. There was one adult male present with 3 or 4 females/juvenile males. The team collected a biopsy sample from an individual that was not the adult male.

The observer team encountered 1 group of false killer whales off the north side of Guam near Ritidian Pt. on 7 September 2015 and collected 1 biopsy sample (Table 4, Fig. 4). There were 25 individuals within the group and none matched to individuals within the photo-identification catalog during a preliminary scan.

The survey team observed a total of 18 turtles off Guam and Rota (Table 5).

Satellite Telemetry

During the summer small-boat surveys off Rota the observer team deployed a SPLASH10 (location-dive) satellite tag on a male bottlenose dolphin (141720) on 2 September. He was a known individual from the photo-identification catalog (TtMI-42) and had been satellite tagged in 2013 (128898) (Hill et al. 2014). The new satellite tag transmitted for 10 days during which the dolphin moved back and forth between Rota and Guam (Fig. 7). During that time, the tag recorded a maximum dive depth of 768 m and multiple other dives between 560 m and 750 m.

PIFSC CRP Shipboard Survey of the Mariana Archipelago

During May–June 2015, the PIFSC CRP conducted a shipboard survey of the entire Mariana Archipelago (Guam to Uracas a.k.a. Farallón de Pajaros) covering areas out to 50 nmi from shore. The tagging team deployed a SPOT5 satellite tag on a false killer whale off Asuncion on 28 May (Fig. 8). The tag transmitted for 30 days during which the false killer whale moved more than 4,600 km. On 21 June, the whale was 1,962 km west of the tag deployment location and 1,500 km outside of the CNMI EEZ; the farthest west that it traveled before turning back to the east (Fig. 8).

Photo-Identification

Photo analysts are currently working on photographs of spinner dolphins collected during the 2014 May–June surveys and have completed the first-round processing and matching for 9 of 17 spinner dolphin encounters. They are also working on photographs of melon-headed whales collected during the 2014 March–April surveys for which they have completed the first-round processing and matching for the first encounter off Saipan on 19 April 2014, and have identified 251 individuals. After matches of individual melon-headed whales are confirmed, photo analysts will create an identification catalog. The analysts have also completed the processing and first-round matching of the melon-headed whale encounter from Guam on 24 April 2014, and have identified 58 individuals. Initial processing and matching of the bottlenose dolphin encounter from 2 September 2015 off Rota is also underway and to date 18 individuals have been identified.

The CRP analyzed pantropical spotted dolphin photos collected off Guam (2010–2014) to assess whether it would be worthwhile to undertake the creation of photo-identification catalogs for the ultimate purpose of mark-recapture analysis and abundance estimation. The photo analysts found that both the quality of the photographs and the lack of distinctiveness of the dorsal fins would hinder a robust mark-recapture analysis. Most individuals in each sighting were represented by fins that would not be usable for mark-recapture due to poor photo-quality, largely a result of fins being partially obscured by water given the typically rough conditions and rapidly-moving animals. Proportion marked values averaged about 0.30 but were not reliable, as on average only 30% of discernible individuals in a group had usable (of sufficient quality) fins. In addition, the proportion marked values were based on a small number of individuals within each encounter (mean of 9, ranging from 1 to 17). The analysts assessed the re-sight potential within the data set by comparing the most distinctive fins across encounters with the assumption that all individuals (very distinctive and less distinctive) would have the same re-sight potential. Of 21 very distinctive fins pulled from the 12 encounters off Guam (2010–2014), 2 individuals were re-sighted. Given these results, the CRP is not pursuing creation of a photo-identification catalog for pantropical spotted dolphins.

Short-finned Pilot Whale Genetics

Appendix I describes the detailed results of nuclear and mitochondrial analysis of short-finned pilot whale samples collected in the Mariana Islands, using samples from the Hawaiian Islands and the eastern tropical Pacific for comparison. Van Cise et al. (submitted) have shown that the Mariana and Hawaiian Islands are inhabited by one type of pilot whale that is mitochondrially distinct from those that inhabit the eastern tropical Pacific, further showing that all 3 of these regions are significantly differentiated, indicating a lack of female gene flow between the regions. These results show that short-finned pilot whales from the 3 regions are

also significantly differentiated in their nuclear DNA, indicating a lack of male gene flow between regions as well. Within the Mariana Islands, Martien et al. (2014) previously showed mitochondrial differentiation between the 3-island group (Saipan, Tinian, Aguijan) and Guam. These results corroborate those findings with additional samples from each area within the Mariana Islands, but did not find any significant differentiation in nuclear DNA, indicating male-mediated gene flow between Guam and the 3-island area. Relatedness analyses of social groups from the Hawaiian Islands that have been stable for over a decade show that short-finned pilot whales tend to be more related to individuals within their own social group than to individuals in other social groups. It is likely that the same is true in the Mariana Islands, with pilot whales choosing to remain in their familial social group rather than leaving the group to find a new one, however the lack of nuclear differentiation between regions suggests that males likely prefer to mate outside their social group, a behavior that has also been described in killer whales (Ford et al. 2011; Pilot et al. 2010).

Acoustic Analysis from Long-term Data sets

Acoustic data collected from the Saipan site for July 2013 through May 2015 and at the Tinian site for June through November 2014 were analyzed for beaked whale signals. Tinian data for 2013–2014 were analyzed and detection details were reported previously (see Hill et al. 2015). Three different beaked whale FM pulse types were detected at Saipan, with expert-based classifications indicating pulses from Blainville's beaked whales (Fig. 9), Cuvier's beaked whales (*Ziphius cavirostris*) (Fig. 10), and the BWC signal type (Fig. 11) possibly belonging to gingko-toothed beaked whales (*M. ginkgodens*). All three signal types were regularly detected throughout the monitoring period at Saipan, although encounters with the BWC type generally occurred at lower numbers. Beaked whale encounters at Tinian were dominated by the FM pulse type produced by Blainville's beaked whales (Fig. 9), with only a single detection of the BWC signal (Fig. 11) at this site. There were no detections of Cuvier's beaked whales in the Tinian deployment.

Diel variability in beaked whale detection was examined across all deployments. The BWC signal type occurred almost exclusively overnight at both sites, while no discernable diel trends were apparent for Blainville's or Cuvier's beaked whale encounters.

Discussion

The 2015 winter and summer small-boat surveys off Saipan, Tinian, Rota and Guam and analysis of acoustic data from Saipan and Tinian represent a continuation of the collaborative effort between the PIFSC's CRP and the U.S. Navy towards a better understanding of the occurrence and distribution of cetaceans in waters off of the southernmost islands of the Mariana Archipelago.

The NMFS (PIFSC) is responsible for the assessment of marine mammal stocks in the Exclusive Economic Zone (EEZ) waters of Guam and CNMI. The U.S. Navy is mandated by permits and Biological Opinions issued under the Marine Mammal Protection Act (MMPA) and the Endangered Species Act (ESA) to monitor cetacean presence within the Mariana Island Range Complex (MIRC). We discuss the preliminary results from the 2015 cetacean surveys in an effort toward answering questions presented within the U.S. Navy's monitoring plan below.

1. What species of beaked whales and other odontocetes occur around Guam and Saipan?
2. Are there locations of greater relative cetacean abundance around Guam and Saipan?

During the 2015 summer (August–September) visual surveys the observer team had only one beaked whale encounter with a group of Blainville's beaked whales seen off Rota. The August 2015 encounter location was much shallower (678 m) than our previous Blainville's beaked whale encounter location off Rota in June 2014 (1200 m), but was farther from shore (15.2 km), closer to Ice Box Reef (Fig. 4).

Acoustic monitoring for beaked whales at Saipan for 2013–2015 and at Tinian for 2014 continues to indicate the occurrence of three species of beaked whales in the region year-round. Blainville's beaked whales are the most frequently detected beaked whale species at both sites, without any apparent peak in occurrence during the year. Unidentified beaked whale BWC is speculated to be ginkgo-toothed beaked whale based on the known and suspected distribution of that species in comparison to the detection of the BWC signal-type. The BWC signal is regularly heard at Saipan, but was heard only once at the Tinian site during the reporting period. Although Cuvier's beaked whales are heard less frequently at Saipan, and not all at Tinian in 2014, the detection range of high-frequency beaked whale signals is likely quite short, such that they may common in other nearby areas, particularly in deeper waters to the east of Tinian.

Patterns of habitat use (depth and distance from shore) by other odontocetes (bottlenose dolphins, spinner dolphins, pantropical spotted dolphins, false killer whales) evident from the 2015 summer (August–September) visual surveys were similar to those described by Hill et al. (2014, 2015).

Although spinner dolphins remained the most frequently sighted species across all survey years, during the 2015 summer they were the second most frequently encountered species (n = 6; 0.29 encounters/100-km effort) after pantropical spotted dolphins (n = 10; 0.48 encounters/100-km effort) (Table 6). This was the result of the distribution of survey effort as the survey team was unable to survey as close to shore during as many days as in previous years because of multiple typhoons and tropical storms within the region that produced high

swell, making conditions dangerous along shore and the outer reefs where spinner dolphins typically occur. The observer team also did not survey around Saipan during summer 2015, where there were numerous spinner dolphin encounters in the past (Hill et al. 2014, 2015). For pantropical spotted dolphins 8 of 10 encounters occurred off Guam during the 2015 summer (Table 4, Fig. 4) unlike the 2014 summer, in which all but 1 encounter was off Rota (Hill et al. 2015).

For the third year in a row the observer team encountered the same group of pygmy killer whales off Guam. A group of 8 individuals was first encountered off Orote Pt. in 2013 (Hill et al. 2014). Then in April 2014, the team encountered the same 8 individuals with 1 YOY near Cocos Island (Hill et al. 2014). On 14 August 2015, the observer team encountered a group of 11 individuals including a neonate. The 9 individuals from the April 2014 encounter were present and the tenth individual was an unmarked calf-sized animal that may have been born after April 2014. The depth of the 2015 encounter location was much deeper (1978 m) and further offshore (9.4 km) than those of the previous years (379 m and 575 m; 1.1 km and 5.1 km). The observer team encountered no other pygmy killer whale groups off Guam in 2010–2015. Off Saipan, the team encountered pygmy killer whale groups but has not been able to get good enough quality photographs for photo-identification. In 2011, a group of 6 pygmy killer whales near Marpi Reef off the northwest side of Saipan ran from the survey vessel. They were 9.9 km offshore in 563 m deep water (Hill et al. 2014). During the 2015 winter, surveys the observer team encountered a group of 6 individuals that were interacting with 2 humpback whales on CK Reef. They were 6.9 km from shore and in 38 m deep water. The fact that the team was focused on the humpback whales along with the ocean conditions and behavior of the pygmy killer whales prevented the collection of photo-id quality photographs. The behavior of both pygmy killer whale groups encountered off Saipan suggests that they are different individuals from those encountered off Guam. Whether the groups encountered off Saipan are the same 6 individuals cannot be determined.

During a PIFSC CRP shipboard survey of the Mariana Archipelago the tagging team deployed a single SPOT5 satellite tag on an adult false killer whale off Asuncion in the northern portion of the Mariana Archipelago. The movements of the tagged false killer whale differed significantly from other false killer whales tagged in previous years. During the 30-day period that the tag was transmitting, the whale spent more time outside of the EEZ boundary than inside of it and traveled 1500 km beyond the western boundary (Fig. 8). The false killer whales tagged in 2013 and 2014 off the southernmost islands of the Archipelago (Guam, Rota, and Tinian) primarily stayed within the boundaries of the EEZ. Some of the previously tagged false killer whales did make excursions away from the islands and even outside of the EEZ, but they also returned to the waters nearshore to the islands (Hill et al. 2014, 2015). The track of

recorded this year suggests that there may be a more transient population of false killer whales that inhabits the Marianas.

3. What is the baseline abundance and population structure of odontocetes which may be exposed to sonar and/or explosives in the near shore areas of Guam, Saipan, Tinian, and Rota?

Although the CRP has produced photo-identification catalogs for spinner dolphins, bottlenose dolphins, short-finned pilot whales, pygmy killer whales, false killer whales, rough-toothed dolphins, and sperm whales, the encounter rate and number of distinctive individuals within each catalog may still be too small to conduct robust abundance analyses. In some cases, catalog size may be used as a proxy for minimum abundance, however we feel that is not yet appropriate in the Marianas as the observer team are adding new individuals to each catalog with each survey. It is not yet possible to determine how many animals may be impacted by explosive or sonar exercises in the region annually. While the areas of underwater detonations and explosive ordnance use off Guam are known and we can begin to assess what species may be exposed, the specific areas of sonar exercises are unknown to us and we are unable to make any evaluation of exposure to cetacean species.

Off Guam, there are 3 Navy training areas where underwater detonations and explosive ordnance use occur. These include the Piti Mine Neutralization Area, the Agat Bay UNDET Area, and the Outer Apra Harbor UNDET Area (Fig. 9). The locations of cetacean encounters during our surveys suggest that exposure to explosive events may occur at Piti and Agat Bay sites. During our 2015 August–September surveys, the observer team encountered groups of pantropical spotted dolphins and a group of pygmy killer whales in the vicinity of these two sites (Fig. 9). To date, the team has not encountered any cetacean groups within Apra Harbor where the Outer Apra Harbor UNDET Area is located (Fig. 9).

4. What is the seasonal occurrence of baleen whales around Guam, Saipan, Tinian, and Rota?

This was the first year that the observer team has encountered any baleen whale during our small-boat surveys in the Marianas. The team specifically conducted surveys during February–March to coincide with the known seasonal occurrence of humpback whales off Saipan and Tinian based on acoustic records (Oleson et al. 2015, Hill et al. 2015), Fulling et al. 2011 and anecdotal reports. The fact that the observer team encountered 4 mom/calf pairs in which the calves were clearly YOYs suggests that the Marianas may be a breeding ground. This could be an important finding if these whales are part of the western North Pacific humpback population that may be designated as Threatened while the other North Pacific humpback

populations are delisted under the Endangered Species Act. We are pursuing avenues to match our fluke photos to existing photo-identification catalogs from the western Pacific population, as well as the genetic analysis of our biopsy samples.

This was also the first year that the observer team encountered Bryde's whales during the small-boat surveys around the southernmost islands of the Mariana Archipelago. Based on these observations alone we cannot say whether their occurrence was related to seasonal movements. Most of our surveys have been during the late spring or early summer and our winter surveys have been limited to shallower nearshore waters. The team did conduct surveys in August–September 2011 and did not encounter Bryde's whales.

Acknowledgements

This project would not have been possible without logistical support and assistance from a great many individuals and organizations. We would like to thank our boat owners, captains and crews: Sam Markos, Ben Sablan, Aesha Sablan, Masao Tembata, Tim Hanley, Chris Weir, and Mark and Lynne Michael.

We would like to thank all of the project assistants and volunteers that assisted with the surveys and provided logistical support for this project: Eric Cruz and Vincent Pangelinan (PIFSC-Guam), Mike Trianni (PIFSC-CNMI), Marlene Reyes (HPU-Guam PIFSC intern), Steve McKagan and Dana Okano (PIRO-CNMI), Tammy Summers (CNMI DFW), Emily Cook (University of Washington), Tyler Vent (Institute for Wildlife Studies), Erik Norris (PIFSC- Honolulu) and Henry Fandel (University of Washington).

All operations in 2015 were conducted under NMFS permit 15240 and CNMI-DFW permit, license no. 03086-2015.

Funding was provided by the Commander, U.S. Pacific Fleet, Environmental Readiness Division, and PIFSC. We would like to thank the individuals at Pacific Fleet (Julie Rivers, Julie Jervey, and Editha Yago) and PIFSC (Martha Kawai) who processed reams of paperwork to ensure that funds were provided for this work.

References

Baumann-Pickering, S., M. A. McDonald, A. E. Simonis, A. Solsona Berga, K. P. B. Merkens, E. M. Oleson, M. A. Roch, S. M. Wiggins, S. Rankin, T. M. Yack and J. A. Hildebrand. 2013. Species-specific beaked whale echolocation signals. Journal of the Acoustical Society of America 134:2293-2301.

Ford, M.J., Hanson, M.B., Hempelmann, J., et al., 2011. Inferred Paternity and Male Reproductive Success in a Killer Whale (Orcinus orca) Population. The Journal of Heredity, 102(5):537–53.

Fulling, G.L., P.H. Thorson, and J. Rivers. 2011. Distribution and abundance estimates for Cetaceans in the waters off Guam and the Commonwealth of the Northern Mariana Islands. Pacific Science 65(3):321-343.

Hancock-Hanser, B.L., Chivers, S., Barid, R., et al., 2013. Targeted multiplex next-generation sequencing: advances in techniques of mitochondrial and nuclear DNA sequencing for population genomics. Molecular Ecology Resources, 13(2):254–68.

Hill, M.C., A.D. Ligon, M.H. Deakos, A.C. Ü, A. Milette-Winfree, A.R. Bendlin, and E.M. Oleson. 2014. Cetacean surveys in the waters of the southern Mariana Archipelago (February 2010 – April 2014). Prepared for the U.S. Pacific Fleet Environmental Readiness Office. PIFSC Data Report DR-14-013. 49 pp. + Appendix.

Hill M.C., A.D. Ligon, E.M. Oleson, K.K. Martien, P.A. Morin, F.I. Archer, S. Baumann-Pickering, A.R. Bendlin, L. Dolar, K.P.B. Merkens, A. Milette-Winfree, A. Rice, K. M. Robertson, J.S. Trickey, A.C. Ü., A.M. Van Cise , and S.M. Woodman. 2015. Cetacean Monitoring in the Mariana Islands Range Complex, 2014. Prepared for the U.S. Pacific Fleet Environmental Readiness Office. PIFSC Data Report DR-15-003. 56 pp. + Appendices.

Martien, K.K., Hill, M.C., Van Cise, A.M., et al., 2014. Genetic diversity and population structure in four species of cetaceans around the Mariana Islands. NOAA Technical Memorandum NMFS-SWFSC-536. 18 pp.

Oleson, E.M., S. Baumann-Pickering, A. Sirovic, K.P. Merkens, L.M. Munger, J.S. Trickey, and P. Fisher-Pool. 2015. Analysis of long-term acoustic datasets for baleen and beaked whales within the Mariana Islands Range Complex (MIRC) for 2010-2013. PIFSC Data Report DR-15-002, 19p.

Pew, J., Muir, P., Wang, J., et al., 2014. related: an R package for analyzing pairwise relatedness from codominant molecular markers. Molecular Ecology Resources, 15:557–561.

Pilot, M., Dahlheim, M.E. & Hoelzel, a. R., 2010. Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (Orcinus orca). Journal of Evolutionary Biology, 23(1):20–31.

Rankin, S., S. Baumann-Pickering, T. Yack and J. Barlow. 2011. Description of sounds recorded from Longman's beaked whale, Indopacetus pacificus. Journal of the Acoustical Society of America 130:EL339-EL344.

Roch, M. A., H. Klinck, S. Baumann-Pickering, D. K. Mellinger, S. Qui, M. S. Soldevilla and J. A. Hildebrand. 2011. Classification of echolocation clicks from odontocetes in the Southern California Bight. Journal of the Acoustical Society of America 129:467-475.
Ryan, W.B.F., S.M. Carbotte, J.O. Coplan, S. O'Hara, A. Melkonian, R. Arko, R.A. Weissel, V. Ferrini, A. Goodwillie, F. Nitsche, J. Bonczkowski, and R. Zemsky (2009), Global Multi-Resolution Topography synthesis, Geochemisty Geophysics Geosystems, 10, Q03014, doi: 10.1029/2008GC002332

Van Cise, A.M., Morin, P.A., Baird, R.W. et al., in review. Redrawing the map: mtDNA provides new insights into the distribution and diversity of short-finned pilot whales in the Pacific Ocean. Submitted to Marine Mammal Science.

Soldevilla, M. S., E. E. Henderson, G. S. Campbell, S. M. Wiggins, J. A. Hildebrand and M. A. Roch. 2008. Classification of Risso's and Pacific white-sided dolphins using spectral properties of echolocation clicks. Journal of the Acoustical Society of America 124:609-624.

Wang, J., 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular ecology resources, 11(1)141–145.

Weatherall, P., K.M. Marks, T. Jakobsson, T Schmitt, S. Tani, J.E. Arndt, MM. Rovere, D. Chayes, V. Ferrini, and R. Wigley. 2015. A new digital bathymetric model of the world's oceans. Earth and Space Science 2:331-345.

Wiggins, S. M. and J. A. Hildebrand. 2007. High-frequency Acoustic Recording Package (HARP) for broad-band, long-term marine mammal monitoring. Pages 551-557 International Symposium on Underwater Technology 2007 and International Workshop on Scientific Use of Submarine Cables & Related Technologies 2007. Institute of Electrical and Electronics Engineers, Tokyo, Japan.

Tables

Table 1: Overview of acoustic data sets analyzed as part of this effort. The duty cycle indicates the number of minutes of recording out of the specified interval.

Deployment	Latitude	Longitude	Depth (m)	Start Effort	End Effort	Duty Cycle
Saipan04	15.3167	145.4583	689	7/23/2013	1/17/2014	5/7
Saipan05	15.3167	145.4583	696	6/18/2014	5/5/2015	5/7
Tinian05	15.0383	145.7517	995	6/16/2014	11/25/2014	5/7

Table 2: Effort summaries for 2015 Marianas winter (February–March) and summer (August–September) surveys.

Date (2015)	Location	Vessel	Survey Description	On Effort Time (h:mm)	On Effort Distance (km)
26-Feb	CNMI-Saipan	Sea Hunter	Saipan–SW loop	4:14	48.7
28-Feb	CNMI-Saipan	Sea Hunter	Saipan–west loop	6:40	82.0
1-Mar	CNMI-Saipan	Sea Hunter	Saipan–west focused on CK Reef	4:38	41.6
2-Mar	CNMI-Saipan	Sea Hunter	Saipan–west focused on CK Reef	3:43	47.0
3-Mar	CNMI-Saipan/ Tinian	Sea Hunter	Saipan/Tinian–west loop	6:36	95.2
6-Mar	CNMI-Saipan	Sea Hunter	Saipan–loop north then to CK Reef	5:53	79.5
7-Mar	CNMI-Saipan	Sea Hunter	Saipan–west CK Reef	4:35	53.0
8-Mar	CNMI-Saipan	Sea Hunter	Saipan–loop north then to CK Reef	6:28	75.1
13-Aug	Guam	Ten27	Hagatna–offshore Hagatna–loop off SW down to Cocos Island	7:40	112.0
14-Aug	Guam	Ten27	Hagatna–offshore loop to SW	6:49	106.8
15-Aug	Guam	Proline	Cabras/Apra Harbor–SW loop 1000-m contour then along shore	2:23	44.2
18-Aug	Guam	Ten27	Hagatna–NW to N offshore then inshore	6:45	116.9

Date (2015)	Location	Vessel	Survey Description	On Effort Time (h:mm)	On Effort Distance (km)
19-Aug	Guam	Ten27	Hagatna–loop south to Orote Pt. along shore then offshore	2:39	40.6
23-Aug	Guam	Ten27	Hagatna–offshore loop south past Orote then north to FAD-2	5:19	89.9
24-Aug	Guam	Ten27	Hagatna–NW offshore loop	4:30	77.9
25-Aug	Guam	Ten27	Hagatna–clockwise offshore circumnavigation	7:35	151.4
26-Aug	Guam	Ten27	Hagatna–SW loop to Galvez Bank	9:13	167.7
28-Aug	CNMI-Rota	Asakaze	Rota West–to Ice Box Reef and counter clockwise circumnavigation	6:41	114.1
29-Aug	CNMI-Rota	Asakaze	Rota West–counterclockwise circumnavigation –offshore	5:58	129.3
30-Aug	CNMI-Rota	Asakaze	Rota West–south side offshore loop	7:06	120.6
31-Aug	CNMI-Rota	Asakaze	Rota West–to Taga Seamount	5:30	113.1
1-Sep	CNMI-Rota	Asakaze	Rota West–loop off south shore	6:17	96.1
2-Sep	CNMI-Rota	Asakaze	Rota West–east-west zigzags off north side	6:32	112.8
3-Sep	Rota to Guam	Asakaze	Rota West to Hagatna via Ice Box Reef and Rota Bank	5:54	111.9
4-Sep	Guam	Ten27	Hagatna–offshore loop to SW	4:10	72.3
5-Sep	Guam	Ten27	Hagatna–NW offshore loop	3:38	58.4
6-Sep	Guam	Ten27	Hagatna–loop SW to W - offshore then along shore	6:04	96.9
7-Sep	Guam	Ten27	Hagatna–NW around Ritidian	7:25	90.6
8-Sep	Guam	Ten27	Hagatna–WNW figure eight	3:55	68.1
			Winter Total:	**42:47**	**522.0**
			Summer Total:	**122:03**	**2091.5**

Table 3: Details of the cetacean encounters during the 2015 Marianas winter (February–March) small-boat surveys.

Date (2015)	Sight	Time (Local)	Common Name	Location	Latitude	Longitude	Depth (m)	Shore Distance (km)	Beaufort	Swell Height (ft)	Total Best	YOY Best	Behavior	No. Biopsy Samples	No. Photos
26-Feb	1	6:46	Humpback whale	CK Reef	15.2108	145.6548	29	6.5	5	4 to 6	1	0	blow	0	0
26-Feb	2	7:38	Humpback whale	CK Reef	15.2304	145.6497	51	7.2	6	6 to 8	1	0	breach	0	0
26-Feb	3	8:24	Humpback whale	CK Reef	15.2236	145.6526	32	6.8	6	6 to 8	2	1	slow travel, evasive	1	121
26-Feb	4	8:47	Bottlenose dolphin	CK Reef	15.2175	145.6548	34	8.3	5	6 to 8	1	0	boat approach, bow ride	0	0
28-Feb	5	8:50	Humpback whale	CK Reef	15.2312	145.6565	37	6.6	6	6 to 8	2	1	mill, evasive	1	254
28-Feb	6	10:08	Humpback whale	CK Reef	15.2113	145.6549	29	6.5	6	6 to 8	1	0	blow	0	0
28-Feb	7	10:37	Humpback whale	CK Reef	15.2034	145.6376	35	8.3	5	6 to 8	2	1	mill	1	266
28-Feb	8	11:18	Humpback whale	CK Reef	15.1891	145.6233	31	9.0	6	6 to 8	2	1	n/a	0	0
1-Mar	9	6:55	Humpback whale	CK Reef	15.2396	145.6611	74	6.4	5	4 to 6	2	1	blow	0	0
1-Mar	10	7:39	Humpback whale	CK Reef	15.2247	145.6554	35	6.5	5	4 to 6	1	0	blow	0	0
1-Mar	11	7:42	Humpback whale	CK Reef	15.2349	145.6570	57	6.6	5	4 to 6	2	1	blow	0	0
1-Mar	12	8:14	Humpback whale	CK Reef	15.2041	145.6531	26	6.6	5	4 to 6	2	1	slow travel	0	0
1-Mar	13	8:49	Humpback whale	CK Reef	15.2145	145.6447	32	7.6	5	4 to 6	1	0	flipper slap, breach, blow	0	0
1-Mar	14	9:26	Humpback whale	CK Reef	15.2302	145.6553	39	6.7	5	6 to 8	1	0	slow travel	0	131

Date (2015)	Sight	Time (Local)	Common Name	Location	Latitude	Longitude	Depth (m)	Shore Distance (km)	Beaufort	Swell Height (ft)	Total Best	YOY Best	Behavior	No. Biopsy Samples	No. Photos
1-Mar	15	9:40	Humpback whale	CK Reef	15.2270	145.6587	32	6.2	5	6 to 8	1	0	blow	0	0
2-Mar	16	6:51	Humpback whale	CK Reef	15.2364	145.6616	63	6.2	5	4 to 6	1	0	blow	0	0
2-Mar	17	7:12	Humpback whale	CK Reef	15.2314	145.6635	58	5.8	4	4 to 6	1	0	blow	0	0
2-Mar	18	7:39	Humpback whale	CK Reef	15.2351	145.6595	57	6.4	5	6 to 8	2	1	blow, dive	0	20
3-Mar	19	6:54	Humpback whale	CK Reef	15.2333	145.6620	58	6.0	5	4 to 6	1	0	blow	0	0
3-Mar	20	7:30	Humpback whale	CK Reef	15.2275	145.6603	37	6.1	5	4 to 6	2	1	slow travel, evasive	0	32
3-Mar	21	8:47	Humpback whale	3.7km W Kilili Bch. Pier	15.2005	145.6803	33	3.7	5	4 to 6	1	0	blow	0	0
6-Mar	22	9:15	Humpback whale	CK Reef	15.2282	145.6570	32	6.4	6	6 to 8	1	0	blow, slow travel, mill	0	63
6-Mar	23	10:09	Humpback whale	CK Reef	15.2168	145.6390	32	8.2	6	6 to 8	2	1	blow, mill	0	82
6-Mar	24	10:50	Humpback whale	CK Reef	15.1760	145.6171	26	9.2	5	6 to 8	2	1	blow, mod trav	0	21
6-Mar	25	11:01	Humpback whale	CK Reef	15.1655	145.6140	33	9.3	6	6 to 8	2	1	blow, slow travel, evasive	1	295
7-Mar	26a	6:56	Humpback whale	CK Reef	15.2286	145.6525	38	6.9	5	4 to 6	1	0	blow, mill, social	1	84
7-Mar	26b	7:05	Pygmy killer whale	CK Reef	15.2250	145.6529	38	6.9	5	4 to 6	6	0	social, porpoise	0	2

Date (2015)	Sight	Time (Local)	Common Name	Location	Latitude	Longitude	Depth (m)	Shore Distance (km)	Beaufort	Swell Height (ft)	Total Best	YOY Best	Behavior	No. Biopsy Samples	No. Photos
7-Mar	26c	6:56	Humpback whale	CK Reef	15.2286	145.6525	33	6.8	5	4 to 6	1	0	blow, social, fluke-up, evasive	0	156
8-Mar	27	8:46	Humpback whale	3.1km W Managaha Is.	15.2449	145.6840	292	4.5	4	4 to 6	1	0	blow, fluke-up, mod travel, evasive	0	35
8-Mar	28	10:28	Humpback whale	CK Reef	15.2395	145.6607	74	6.4	4	4 to 6	1	0	blow	0	0
8-Mar	29	11:09	Humpback whale	CK Reef	15.2258	145.6544	33	6.7	5	4 to 6	2	1	blow, slow travel, evasive	0	100
													Total:	**5**	**1662**

Table 4: Details of the cetacean encounters during the 2015 Marianas summer (August–September) small-boat surveys.

Date (2015)	Sight	Time (Local)	Common Name	Location	Latitude	Longitude	Depth (m)	Shore Distance (km)	Beaufort	Swell Height (ft)	Total Best	YOY Best	Neonates Best	Behavior	No. Biopsy Samples	No. Tags	No. Photos
13-Aug	1	12:30	Pantropical spotted dolphin	Guam	13.5960	144.7673	757	6.6	4	2 to 4	103	4	3	leap, mod trav, boat approach, bow ride	0	0	488
14-Aug	2	8:55	Pygmy killer whale	Guam	13.3704	144.5541	1978	9.4	4	2 to 4	11	1	0	slow travel, low swim, evasive	1	0	693
15-Aug	3	8:53	Spinner dolphin	Guam	13.2801	144.6585	40	0.4	4	0 to 2	22	0	0	boat approach, bow ride, leap, mill	0	0	16
18-Aug	4	7:27	Pantropical spotted dolphin	Guam	13.5495	144.7299	885	6.8	2	4 to 6	52	0	1	boat approach, bow ride	0	0	75
18-Aug	5	9:40	Pantropical spotted dolphin	Guam	13.7038	144.9014	433	6.7	3	4 to 6	59	0	0	fast travel, evasive, porpoise	0	0	9
18-Aug	6	11:55	Spinner dolphin	Guam	13.6139	144.9087	28	0.5	3	0 to 2	3	0	0	rest	0	0	2
19-Aug	7	6:45	Spinner dolphin	Guam	13.4876	144.7372	89	0.9	2	2 to 4	1	0	0	porpoise	0	0	0
26-Aug	8	8:43	Pantropical spotted dolphin	Guam	13.3008	144.4962	1395	15.7	2	4 to 6	121	0	2	leap, mod trav	3	0	1000
28-Aug	9	7:23	Blainville's beaked whale	CNMI–Rota	14.0295	145.0139	678	15.2	1	2 to 4	5	0	0	slow travel	1	0	361
28-Aug	10	10:06	Bryde's whale	CNMI–Rota	14.0060	145.1133	687	12.4	1	2 to 4	1	0	0	slow travel, evasive	1	0	196
29-Aug	11	11:33	Spinner dolphin	CNMI–Rota	14.1252	145.1600	31	0.7	1	0 to 2	28	2	0	rest, boat approach, bow ride, mod trav	0	0	21
30-Aug	12	7:04	Bottlenose dolphin	CNMI–Rota	14.0421	145.0697	846	10.1	1	2 to 4	27	0	1	mill, feed, slow travel	9	0	818

Date (2015)	Sight	Time (Local)	Common Name	Location	Latitude	Longitude	Depth (m)	Shore Distance (km)	Beaufort	Swell Height (ft)	Total Best	YOY Best	Neonates Best	Behavior	No. Biopsy Samples	No. Tags	No. Photos
30-Aug	13	11:20	Sei/Bryde's whale	CNMI–Rota	13.9173	145.2281	1918	21.9	1	2 to 4	1	0	0	blow, evasive	1	0	184
31-Aug	14	6:48	Pantropical spotted dolphin	CNMI–Rota	14.1582	145.0997	933	4.2	2	2 to 4	43	0	0	boat approach, bow ride, chase, mill, leap	0	0	42
1-Sep	15	9:01	Spinner dolphin	CNMI–Rota	14.1936	145.2951	99	0.7	4	6 to 8	90	1	0	boat approach, bow ride, wave ride	0	0	263
1-Sep	16	10:22	Bottlenose dolphin	CNMI–Rota	14.1134	145.2070	67	0.3	4	2 to 4	21	1	0	slow travel	0	0	409
1-Sep	17	11:59	Unid. Whale	CNMI–Rota	14.1307	145.2427	447	1.3	1	2 to 4	1	0	0	blow	0	0	0
2-Sep	18a	8:54	Pantropical spotted dolphin	CNMI–Rota	14.2306	145.1289	967	7.7	0	2 to 4	16	0	0	boat approach, bow ride, fast travel	0	0	118
2-Sep	18b	9:10	Bottlenose dolphin	CNMI–Rota	14.2383	145.1262	1048	8.6	0	2 to 4	3	0	0	mod trav, chase	1	0	105
2-Sep	19	11:52	Bottlenose dolphin	CNMI–Rota	14.1707	145.1591	18	0.3	4	2 to 4	14	0	0	slow travel	1	1	524
3-Sep	20	9:06	Bryde's whale	Guam–Rota Bank	13.8328	144.9910	487	23.9	3	2 to 4	1	0	0	feed, evasive	0	0	70
3-Sep	21	12:03	Pantropical spotted dolphin	Guam	13.6572	144.8131	499	4.2	3	2 to 4	25	0	0	leap, boat approach, bow ride, slow travel	0	0	135
4-Sep	22	13:37	Pantropical spotted dolphin	Guam	13.4614	144.6093	755	1.7	4	2 to 4	76	1	0	leap, boat approach, bow ride	0	0	261
4-Sep	23	14:49	Pantropical spotted dolphin	Guam	13.5295	144.6829	1117	6.4	2	2 to 4	13	2	0	leap, slow travel	0	0	125

Date (2015)	Sight	Time (Local)	Common Name	Location	Latitude	Longitude	Depth (m)	Shore Distance (km)	Beaufort	Swell Height (ft)	Total Best	YOY Best	Neonates Best	Behavior	No. Biopsy Samples	No. Tags	No. Photos
5-Sep	24	7:29	Bryde's whale	Guam	13.5989	144.6797	859	13.8	4	2 to 4	1	0	0	slow travel, evasive	1	0	188
6-Sep	25	7:54	Pantropical spotted dolphin	Guam	13.5079	144.6042	1906	6.0	3	2 to 4	90	2	1	leap, mod trav, porpoise	0	0	359
6-Sep	26	11:34	Spinner dolphin	Guam	13.5288	144.7974	92	0.7	4	2 to 4	101	6	2	mill, boat approach, bow ride	0	0	374
7-Sep	27	9:08	False killer whale	Guam	13.6695	144.9116	389	4.4	3	2 to 4	25	0	0	fast travel, boat approach, mill, feed, porpoise, evasive	1	0	1174
														Total:	**20**	**1**	**8010**

Table 5: Turtle sightings during the 2015 Marianas winter (February–March) and summer (August–September) small-boat cetacean surveys.

Date (2015)	Time (Local)	Island	Lat	Long	Description
26-Feb	10:36	Saipan	15.2136	145.6934	Green Turtle-large (> 2.5 ft)
28-Feb	13:17	Saipan	15.2268	145.7040	Green Turtle-med (1.5–2.5 ft)
1-Mar	11:22	Saipan	15.2284	145.7126	Turtle-med (1.5–2.5 ft)
2-Mar	10:12	Saipan	15.2176	145.6918	Turtle-med (1.5–2.5 ft)
2-Mar	10:12	Saipan	15.2188	145.6930	Turtle-large (> 2.5 ft) x2
3-Mar	12:45	Saipan	15.1674	145.6873	Turtle-med (1.5–2.5 ft)
3-Mar	13:03	Saipan	15.2091	145.6940	Green Turtle-large (> 2.5 ft)
3-Mar	13:09	Saipan	15.2218	145.7001	Turtle-large (> 2.5 ft)
3-Mar	13:15	Saipan	15.2274	145.7114	Green Turtle-med (1.5–2.5 ft)
3-Mar	13:19	Saipan	15.2264	145.7207	Green Turtle-large (> 2.5 ft)
6-Mar	12:36	Saipan	15.2270	145.7194	Green Turtle-large (> 2.5 ft)
7-Mar	11:10	Saipan	15.2181	145.6922	Turtle-small (< 1.5 ft)
7-Mar	11:11	Saipan	15.2193	145.6934	Turtle-med (1.5–2.5 ft)
7-Mar	11:17	Saipan	15.2266	145.7032	Green Turtle-small (< 1.5 ft) x2
7-Mar	11:25	Saipan	15.2256	145.7221	Turtle-med (1.5–2.5 ft)
7-Mar	11:26	Saipan	15.2239	145.7227	Green Turtle-small (< 1.5 ft)
15-Aug	9:14	Guam	13.2901	144.6542	Green Turtle-small (< 1.5 ft)
18-Aug	13:12	Guam	13.5942	144.8335	Green Turtle-large (> 2.5 ft)
1-Sep	9:48	Rota	14.1597	145.2839	Green Turtle-small (< 1.5 ft)
1-Sep	9:53	Rota	14.1515	145.2712	Green Turtle-large (> 2.5 ft) x2
1-Sep	9:57	Rota	14.1509	145.2615	Green Turtle-small (< 1.5 ft)
1-Sep	12:45	Rota	14.1328	145.1533	Green Turtle-med (1.5–2.5 ft)
1-Sep	12:55	Rota	14.1312	145.1386	Green Turtle-small (< 1.5 ft)
6-Sep	9:54	Guam	13.4102	144.6515	Turtle-med (1.5-2.5 ft)
6-Sep	9:56	Guam	13.4120	144.6461	Green Turtle-med (1.5–2.5 ft) Green Turtle-large (> 2.5 ft)
6-Sep	10:50	Guam	13.4769	144.6964	Turtle-large (> 2.5 ft)
6-Sep	11:26	Guam	13.5106	144.7872	Turtle-med (1.5–2.5 ft)
6-Sep	11:27	Guam	13.5114	144.7901	Turtle-med (1.5–2.5 ft)
6-Sep	11:31	Guam	13.5201	144.7983	Green Turtle-large (> 2.5 ft)
6-Sep	11:32	Guam	13.5214	144.7990	Turtle-large (> 2.5 ft)
6-Sep	12:38	Guam	13.4856	144.7557	Green Turtle-med (1.5–2.5 ft)
8-Sep	8:41	Guam	13.4782	144.6975	Turtle-med (1.5–2.5 ft)

Table 6: Species encounter summary including encounter rate (No. encounters/100 km effort), depth (m) and distance from shore (km) for 2015 Marianas summer (August–September) small-boat cetacean surveys. Includes total encounters and overall encounter rates across all survey years (2010–2015) for species encountered during summer 2015 (17,093.2 km total survey distance).

Species	No. Species Encounters (Total 2010–2015*)	Encounters/ 100km Effort (Overall 2010–2015*)	Median Depth (m) (min-max)	Median Shore Distance (km) (min-max)
Pantropical spotted dolphin	10 (37)	0.48 (0.22)	909 (433–1906)	6.5 (1.7–15.7)
Spinner dolphin	6 (108)	0.29 (0.63)	64 (28–99)	0.7 (0.4–0.9)
Bottlenose dolphin	4 (24)	0.19 (0.14)	457 (18–1048)	4.4 (0.3–10.1)
Bryde's whale	3 (3)	0.14 (0.02)	687 (487–859)	13.8 (12.4–23.9)
Blainville's beaked whale	1 (2)	0.05 (0.01)	678	15.2
False killer whale	1 (6)	0.05 (0.04)	389	4.4
Pygmy killer whale	1 (4)	0.05 (0.02)	1978	9.4
Sei/Bryde's whale	1 (1)	0.05 (0.01)	1918	21.9
Unid. Whale	1 (1)	0.05 (0.01)	447	1.3
Total:	**28**	**1.34**		

*2015 winter effort not included in calculations because the effort targeted humpback whales.

Figures

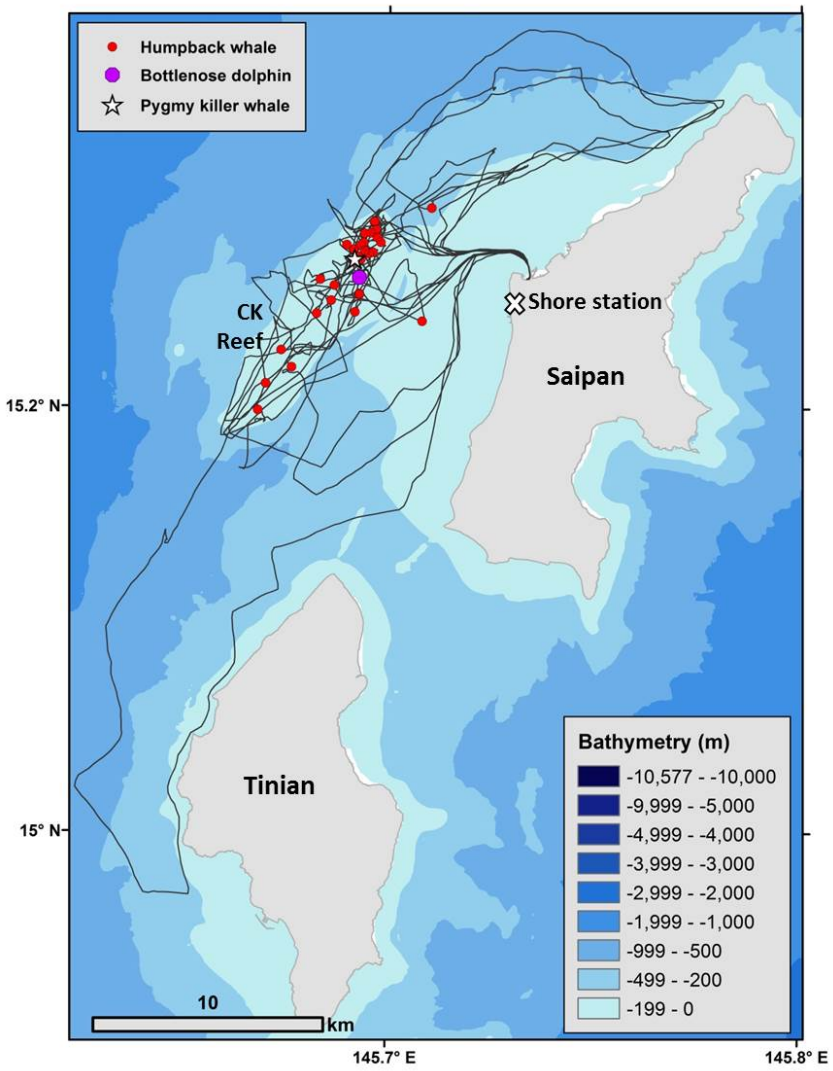


Figure 1: Tracklines and cetacean sighting locations during the 2015 Marianas winter (February–March) small-boat surveys.

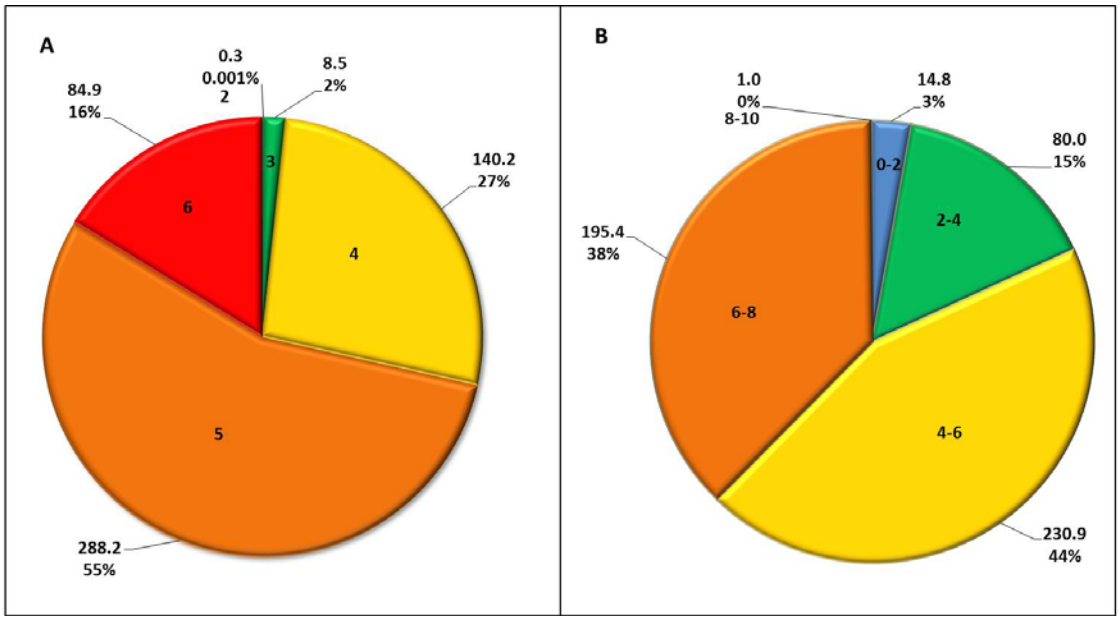


Figure 2: Effort by (A) Beaufort sea state and (B) swell height (ft) during the 2015 Marianas winter (February–March) small-boat cetacean surveys.

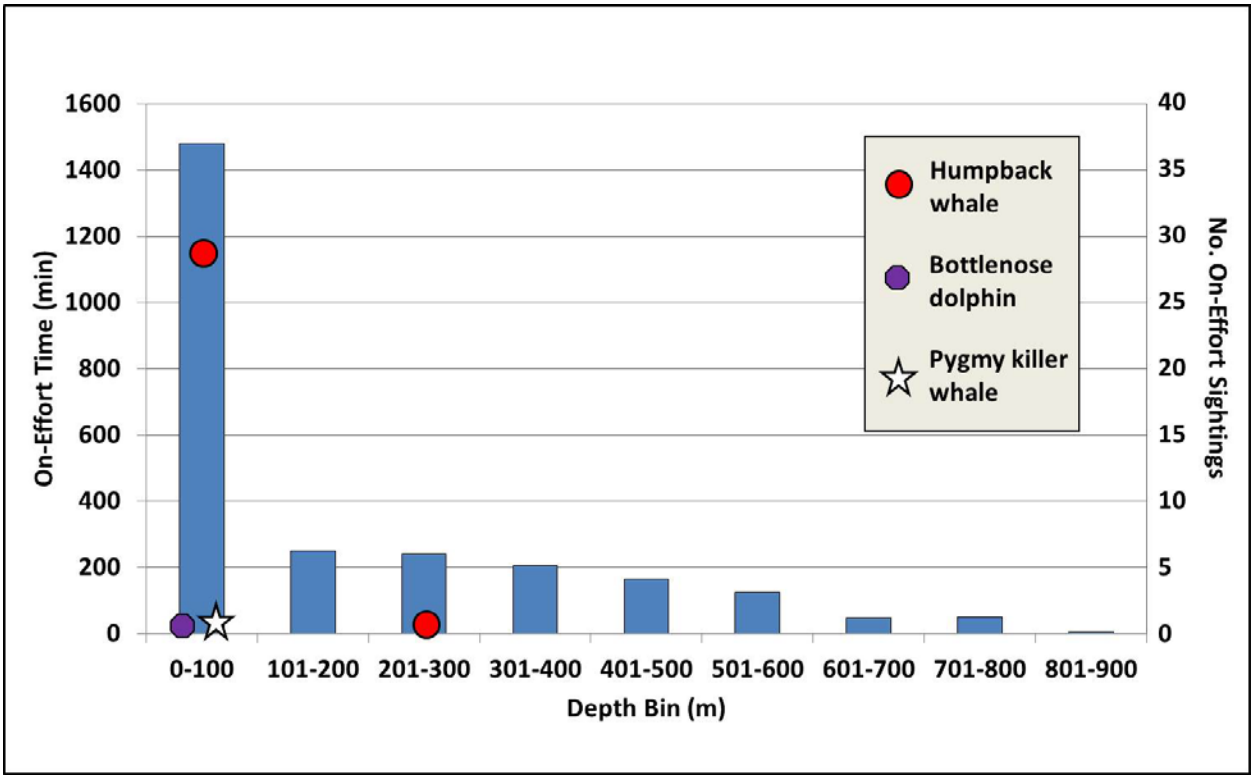


Figure 3: Effort and sightings by depth during the 2015 Marianas winter (February–March) small-boat cetacean surveys.

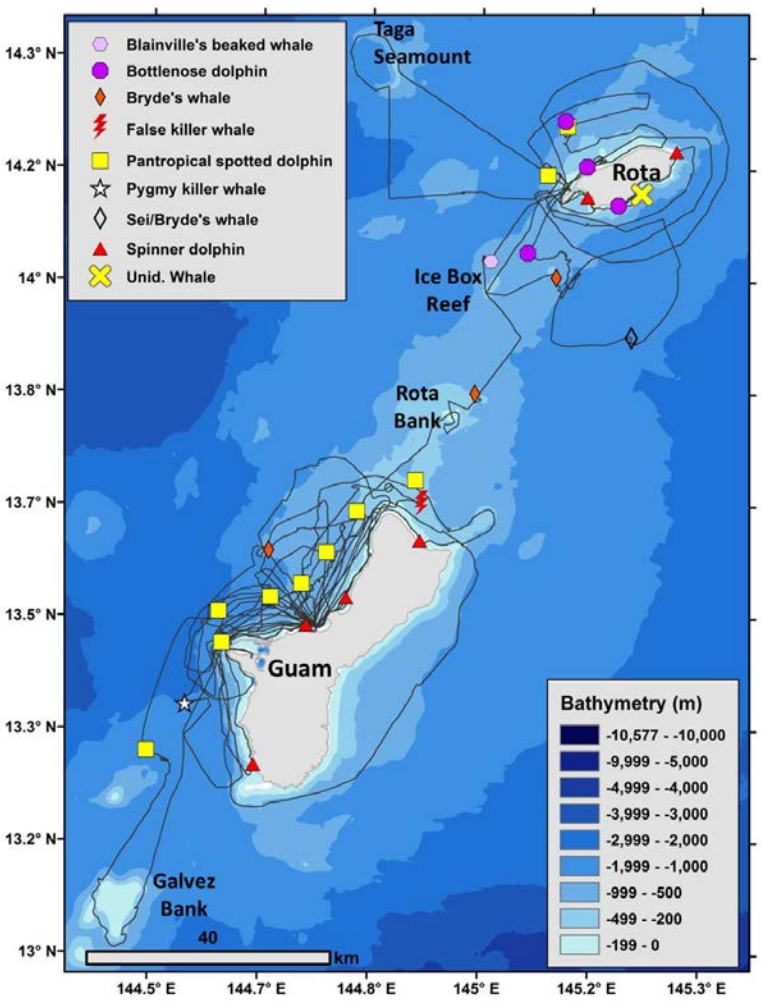


Figure 4: Tracklines and cetacean sighting locations during the 2015 Marianas summer (August–September) small-boat surveys.

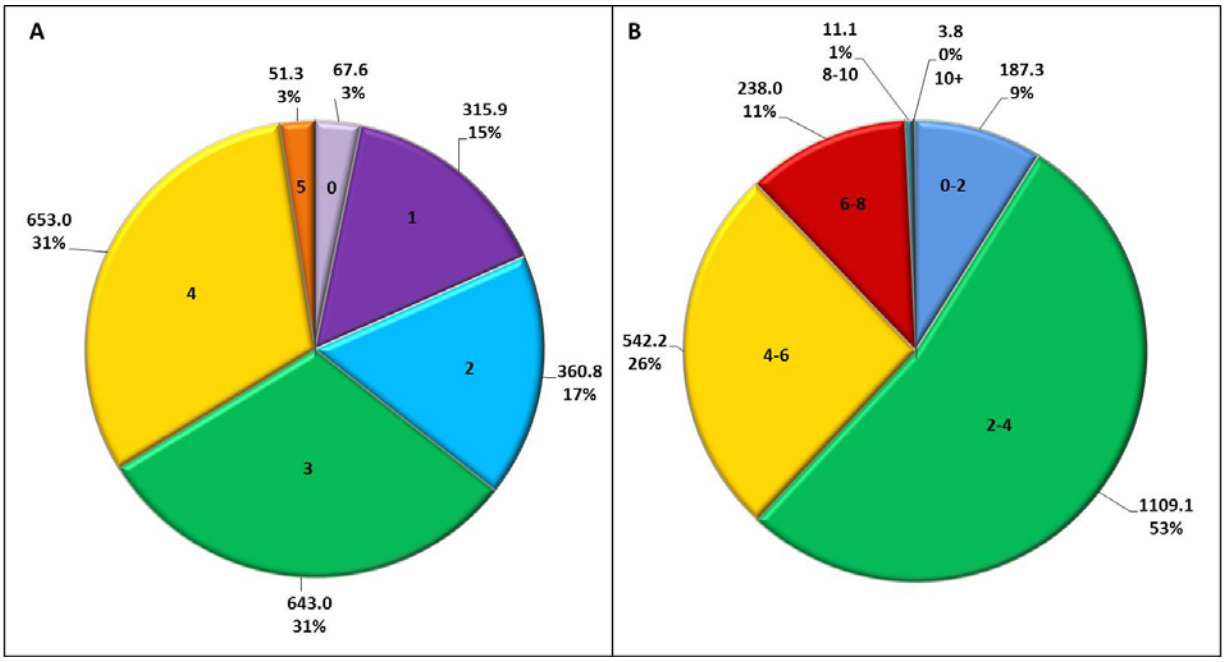


Figure 5: Effort by (A) Beaufort sea state and (B) swell height (ft) during the 2015 Marianas summer (August–September) small-boat cetacean surveys.

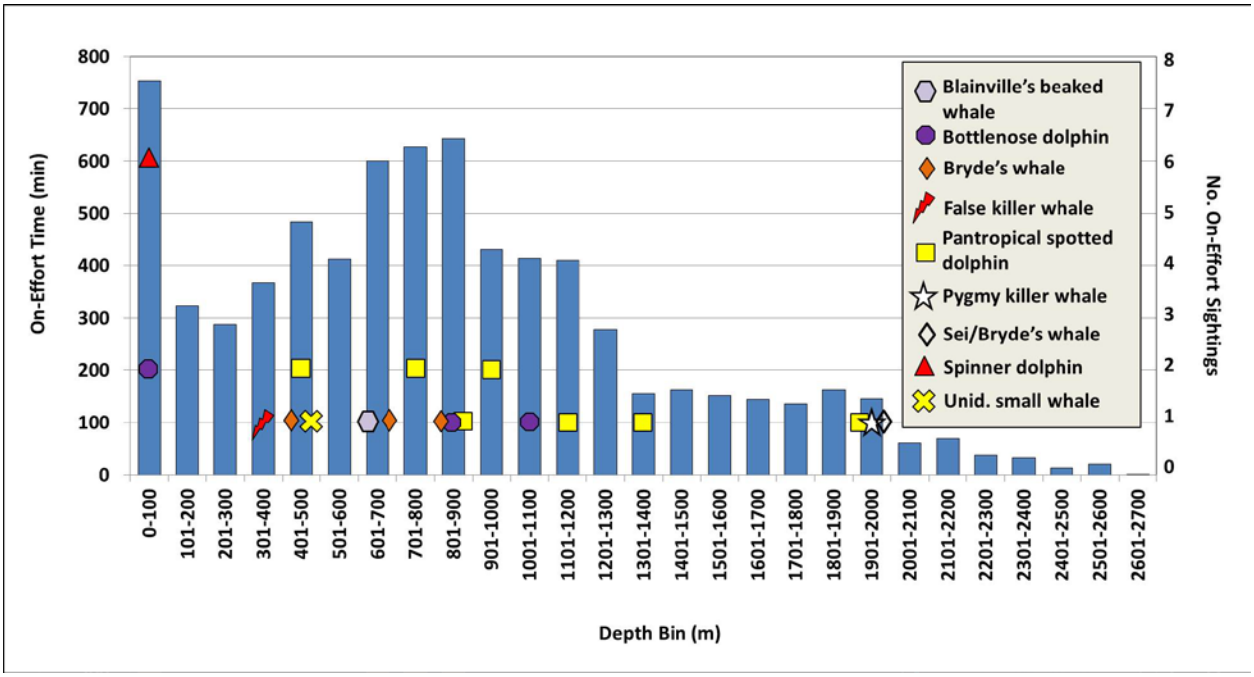


Figure 6: Effort and sightings by depth during the 2015 Marianas summer (August–September) small-boat cetacean surveys.

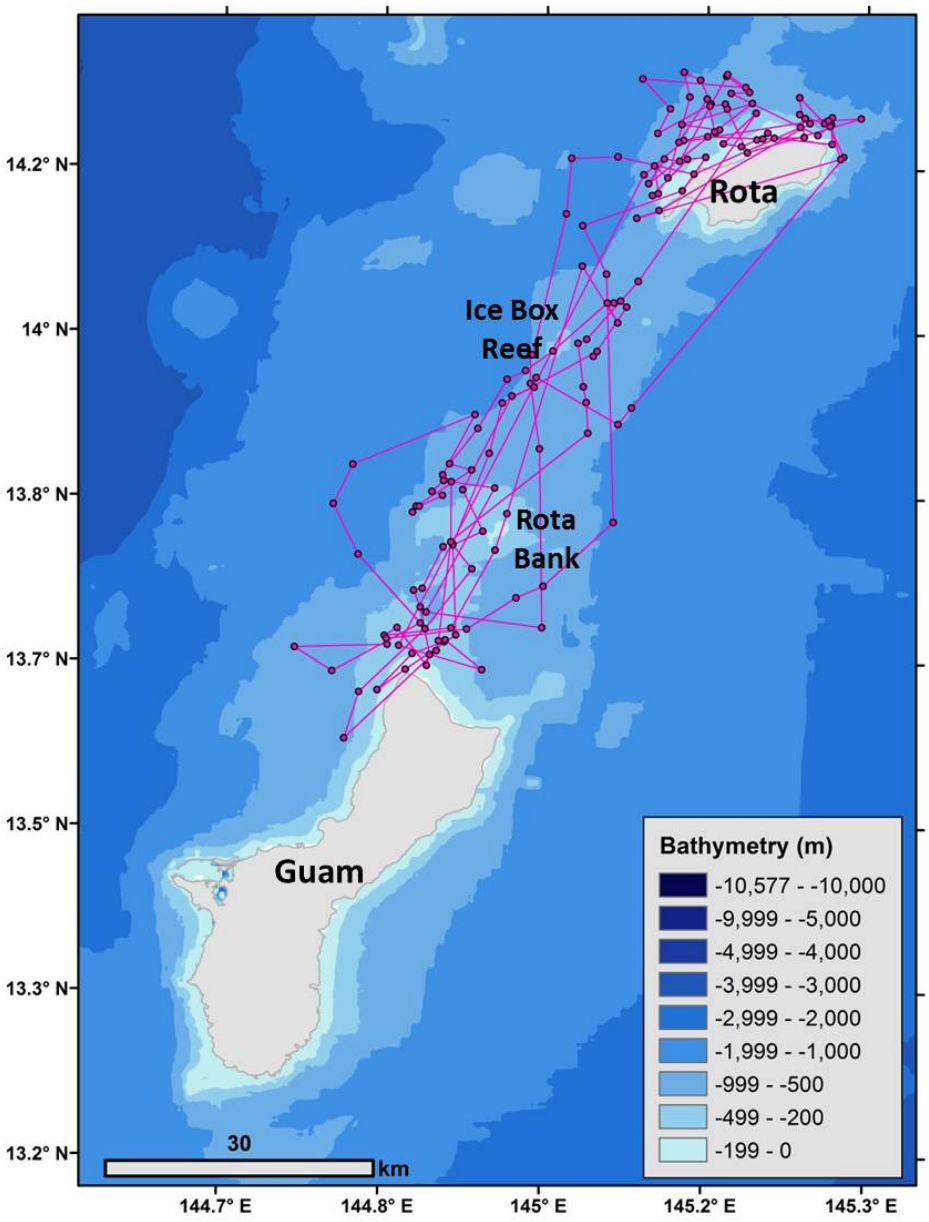


Figure 7: Tracks of a SPLASH10 satellite tag (141720) deployed on a male bottlenose dolphin off Rota on 2 September 2015 (10-day duration).

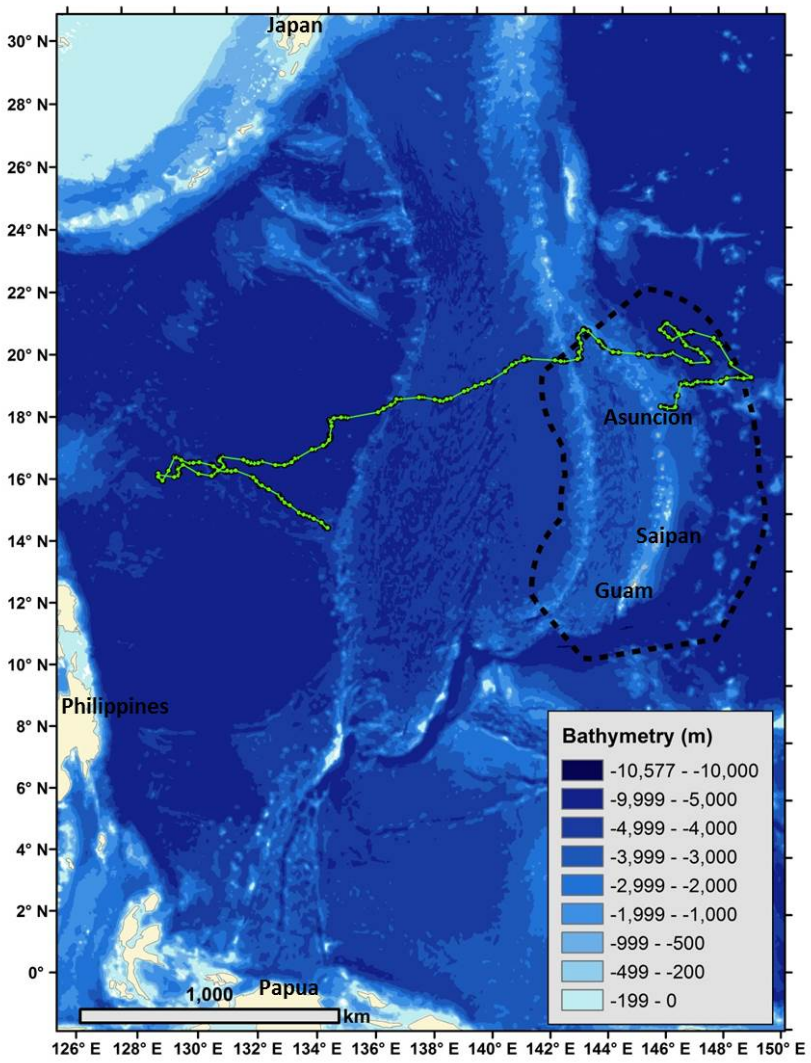


Figure 8: Tracks of a SPOT5 satellite tag (128905) deployed on a false killer whale off Asuncion on 28 May 2015 (30-day duration) during PIFSC CRP shipboard survey.

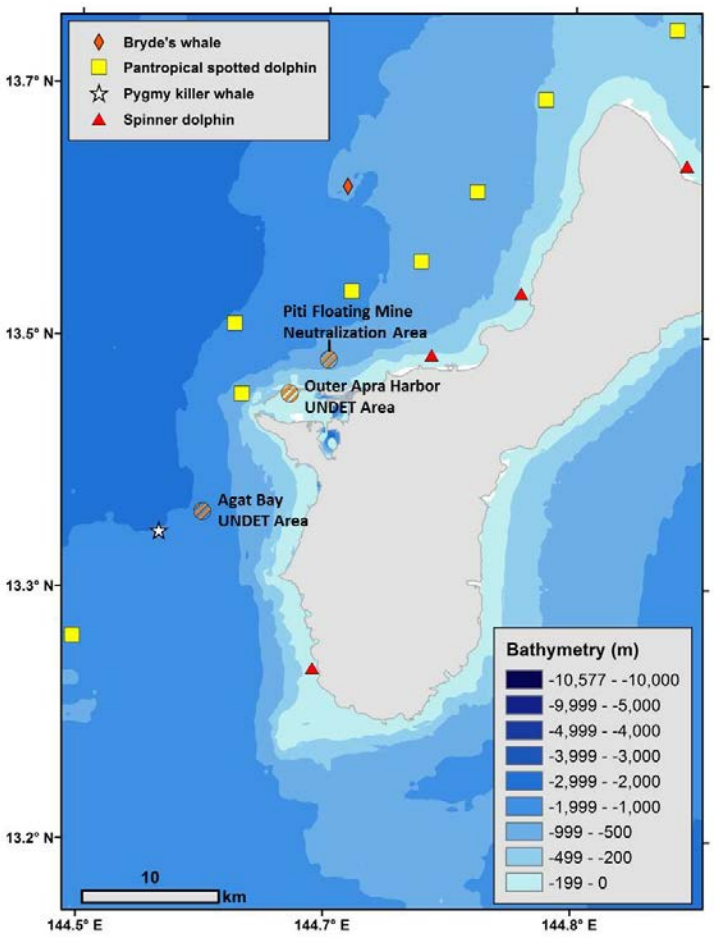


Figure 9: 2015 cetacean encounter locations off Guam and Navy underwater detonation sites.

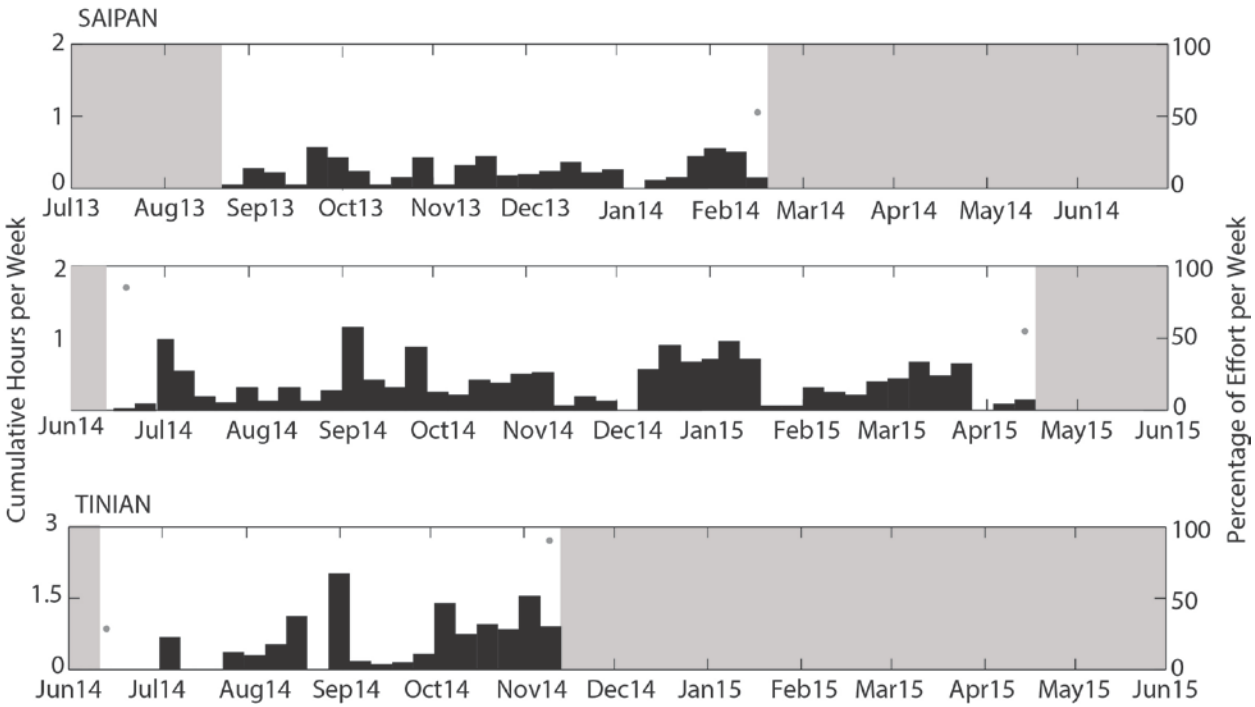


Figure 10. Weekly occurrence of Blainville's beaked whales at Saipan in 2013–2015 (upper and middle panel) and at Tinian in 2014–2015 (bottom panel). Note that month axis extends from June through May of the following year. Periods with no data are shown as gray boxes. Gray dots indicate less than a full week of available data.

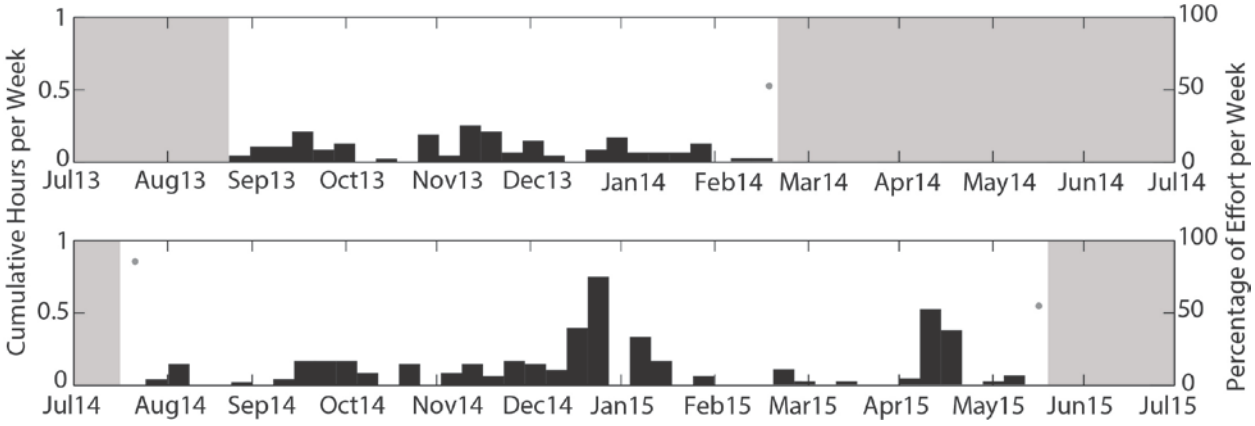


Figure 11. Weekly occurrence of Cuvier's beaked whales at Saipan in 2013–2015. Note that month axis extends from June through May of the following year. Periods with no data are shown as gray boxes. Gray dots indicate less than a full week of available data. There were no Cuvier's beaked whale detections in the Tinian data set.

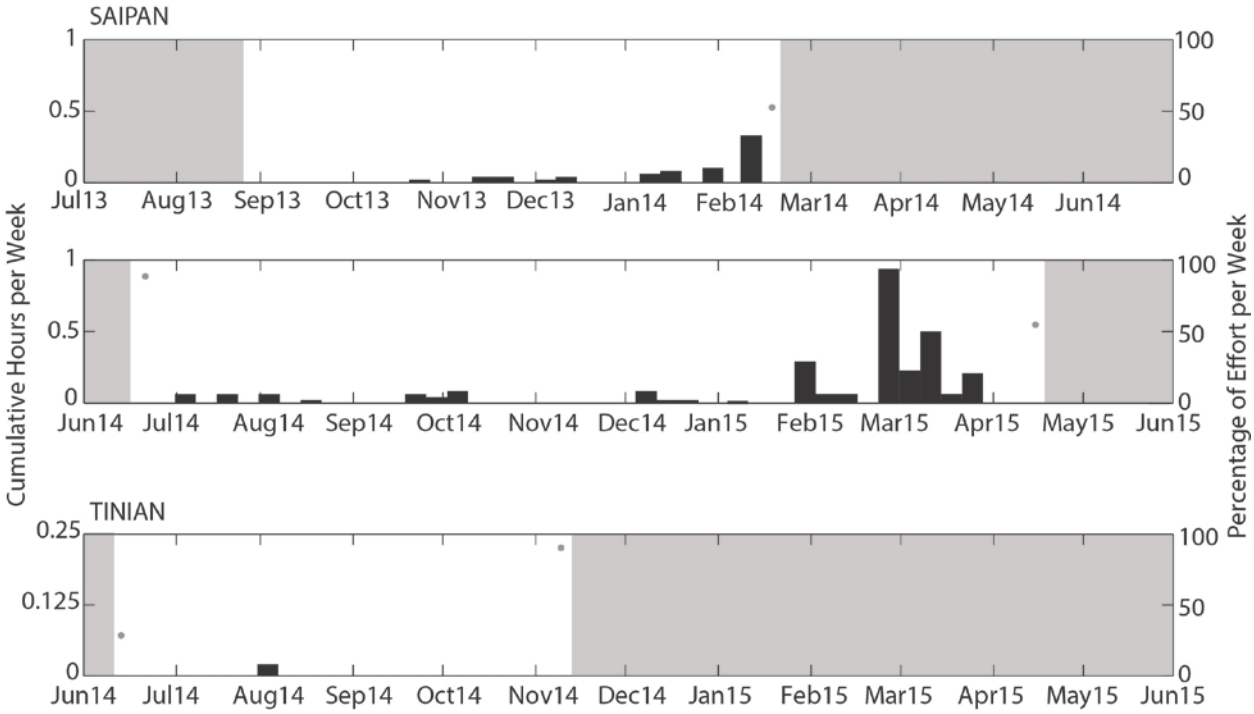


Figure 12. Weekly occurrence of unidentified beaked whale BWC at Saipan in 2013–2015 (upper and middle panel) and at Tinian in 2014–2015 (bottom panel). Note that month axis extends from June through May of the following year. Periods with no data are shown as gray boxes. Gray dots indicate less than a full week of available data.

Appendix I: Relatedness and genetic structure in island-associated and pelagic short-finned pilot whales

Amy M. Van Cise[1,2], Phillip A. Morin[2,1], Robin W. Baird[3], Erin M. Oleson[4], Karen K. Martien[2]

1. Scripps Institution of Oceanography, 8622 Kennel Way, La Jolla, CA 92037
2. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 8901 La Jolla Shores Drive, La Jolla CA 92037
3. Cascadia Research Collective, 218 1/2 4th Ave W, Olympia, WA 98501
4. Pacific Islands Fisheries Science Center, National Marine Fisheries Service, NOAA, 1845 Wasp Boulevard, Building 176, Honolulu, HI 96818

Abstract

Broadly distributed species often exhibit genetic structure caused by habitat preferences or social structure. Here we examine regional and local genetic structure in Pacific short-finned pilot whales using a combination of mtDNA haplotypes and SNP genotypes of samples from the Mariana Islands, Hawaiian Islands and the eastern tropical Pacific (ETP). Preliminary analyses indicate a lack of male- or female-mediated gene flow between these three regions, and suggest a greater degree of population structure within island associated populations than pelagic populations. Within the Mariana Islands, mitochondrial differentiation was found between Saipan and Guam/Rota, but no nuclear differentiation was found between any region, suggesting that gene flow is driven by males of the species. High relatedness within social groups suggests the possibility of socially-mediated population structure, which will be a topic of future research and analysis.

Introduction

Ocean basins have few barriers to distribution, and indeed many marine vertebrates have migration routes that cover entire basins over the course of a year. Most odontocetes, however, exhibit genetic structure due to localized habitat use throughout the range of their distribution. In the case of an apparent lack of physical barriers, cryptic genetic structure is often driven by less obvious mechanisms, such as environmental preferences (e.g., sea surface temperature (SST), thermocline depth), dietary specialization, or social structure. Moreover, different habitats, such as coastal or island-associated and pelagic marine habitats, can facilitate different patterns of genetic structure within and among populations of the same species.

Short-finned pilot whales are distributed throughout the Pacific Ocean in tropical and temperate waters. Although they are most often observed along shelf breaks, over depths of 1500 m, they are known to occupy pelagic waters in areas such as the eastern tropical Pacific (ETP) (Baird et al. 2013; Hamilton et al. 2009). Two morphologically and genetically distinct types have been described, with spatio-temporally non-overlapping distributions (Kasuya *et al.* 1988; Oremus *et al.* 2009, Van Cise et al. *in review*). Mitogenomic data indicate that a third genetically distinct clade overlaps the distributions of the first two (Morin *et al.* 2015), although there are no morphological data to support or refute this hypothesis. The Naisa type short-

finned pilot whale inhabits Hawai'i, SE Asia and southern Japan seas, and has also been documented in the Indian Ocean (Van Cise et al. *in review*). The Shiho type short-finned pilot whale is largely distributed throughout the eastern Pacific Ocean, with a smaller population off the coast of northern Japan. The third, largely undescribed type (inferred only from mtDNA sequence data), is found in the South Pacific Islands, the Mariana Islands, SE Asia, and extends eastward into the pelagic ETP, although its distribution is limited in this region (Morin et al. 2015).

Pilot whales are a gregarious species, with a mean group size of 20–25 animals (e.g. Baird *et al.* 2013; Barlow 2006). A long-term study in the Hawaiian Islands has identified social groups that are stable for decades or more (Mahaffy et al. 2015). Like other social cetaceans, such as killer whales and sperm whales, these social groups of pilot whales will interact with other groups, but retain their original group membership. This stable social structure may be an important driver of local population structure in short-finned pilot whales, yet it is currently unknown whether these groups comprise related individuals or offspring from many families.

Understanding the importance of local social structure could help us understand local genetic divergence, for example, in the Mariana Islands, where Martien et al. (2014a) reported genetic divergence in resident pilot whales that was not anticipated based on existing data on animal movements. Analysis of mtDNA sequences revealed divergence between Guam/Rota and Saipan, suggesting the existence of two populations (Martien et al. 2014a). This seemed to contradict photo ID and satellite tag data indicating movement between the islands, and suggested the need for an analysis of genomic DNA to test for male-mediated gene flow between the islands.

Here we augment our current understanding of broad genetic structure in the Pacific Ocean by comparing mtDNA sequences and single nucleotide polymorphism (SNP) genotypes to examine population structure on a basin-wide scale, using samples from the Mariana Islands, the Hawaiian Islands and the ETP. We further examine mtDNA and SNP divergence within each region to better understand local population structure and divergence in these regions, as well as examining the role of male- vs female-mediated gene flow. In the Hawaiian Islands, where social structure is well-documented, we test for relatedness among social groups in order to determine whether social structure is a potential driver of genetic diversity in short-finned pilot whales.

Methods

Data collection

DNA sequences from *G. macrorhynchus* were generated from samples in the Southwest Fisheries Science Center (SWFSC) Marine Mammal and Turtle Molecular Research Sample Collection. Samples were stored at -80°C, or fixed in either a salt-saturated 20% DMSO solution or 100% ethanol and permanently archived in a -20°C freezer. The archived skin samples from 231 short-finned pilot whales collected from the Mariana Islands (n = 48), Hawaiian Islands (n = 144) and the ETP (n = 39) were used in SNP analysis. Mariana Island and ETP samples were chosen to maximize a distribution over all encounters in the region, with 1–2 samples chosen per mtDNA haplotype per encounter. In the absence of nDNA data to control for variation in relatedness among sample sets, we used selection of one individual per haplotype to minimize overrepresentation of relatives within sampled groups, but this could have the effect of

overestimating genetic diversity measures. In the Hawaiian Islands, known social groups were heavily sampled in order to test for relatedness; additional samples were chosen randomly, with consideration given to ensuring a representative sample was selected from each stratum.

DNA sequencing and assembly

DNA was extracted from skin and muscle samples using a sodium chloride precipitation protocol (Miller *et al.* 1988), Qiagen DNeasy Blood and Tissue Kit (#69506, Qiagen, Germantown, MD, USA) or a phenol-chloroform protocol (Sambrook *et al.* 1989). The hypervariable mtDNA control region was amplified and sequenced in two parts of approximately 420 bp and 560 bp, with approximately 20 bp of overlap between the two sequences. Primers, PCR and sequencing methods have been previously described by Martien *et al.* (2014b). The resulting combined sequence was 962 bp, and was assembled using SEQED, version 1.0.3 (ABI), Sequencher software (versions 4.1 and 4.8; Gene Codes, Ann Arbor, MI, USA) or Geneious (version 6.1.5, Biomatters Ltd, Auckland, New Zealand).

Mitochondrial sequences were aligned using a MAFFT alignment with default parameters (Scoring Matrix: 200PAM/k = 2, Gap open penalty: 1.53, Offset value: 0.123) in the Geneious software package (Katoh & Kuma 2002). Once the alignment was completed, sequences were re-examined. Any haplotypes represented by only a single sequence or haplotypes with a single base-pair difference from the most similar haplotype were reviewed for accuracy. Unique haplotypes were repeat sequenced in order to ensure the accuracy of the sequence.

Sequencing of 78 targeted nuclear loci for SNP analysis was completed using a custom capture enrichment array designed at SWFSC, followed by highly-parallel sequencing (Hancock-Hanser *et al.* 2013). Four libraries of genomic DNA were prepared using protocols described in Meyer and Kircher (2010) and Hodges *et al.* (2009), with modifications described in Hancock-Hanser *et al.* (2013). Up to 400 ng of extracted DNA in 80 µL total volume was sonicated using a Bioruptor UCD-200 (Diagenode). Blunt-ends of the DNA were repaired using 20 µL of the sonicated product, adaptors were ligated to the DNA, and indexes were added to each sample before all samples were pooled and hybridized to the capture array. The hybridized product was amplified, then sequenced (1 x 100 bp) on Illumina HiSeq or NextSeq instruments by The DNA Array Core Facility (The Scripps Research Institute, La Jolla, CA).

Nuclear sequences were assembled to a reference sequence and SNPs identified using scripts developed at SWFSC (Dryad data repository doi:10.5061/dryad.cv35b) in the R computing environment (R Development Core Team 2006). The cutoff for calling a genotype at any position was set to five reads for homozygous positions and 10 reads for heterozygous positions. Candidate SNPs were manually chosen at each locus. Of the candidate SNPs, those with coverage at fewer than 35% of samples were removed to generate the final SNP set. Samples with coverage at fewer than 50% of the final SNP set were removed from the analysis. SNP genotypes were used to identify sample replicates using a QAQC analysis in the strataG package for R, and samples determined to be replicates were removed from the data set prior to analysis. Loci with multiple SNPs were phased based on allele frequencies in the three regional strata, with a phase cutoff probability of 0.5, to generate a single genotype per sample at each locus for population differentiation and STRUCTURE analyses (Morin et al. 2012). For

analysis of relatedness within Hawaiian social groups, the highest heterozygosity SNP at each locus was chosen for the analysis.

Data analysis

Samples were stratified in a hierarchical manner. At the broadest stratification level of geographic regions, samples were grouped geographically into Mariana Islands, Hawaiian Islands and ETP strata. The Hawaiian Islands stratum was subsampled to a sample size comparable to that from other strata for molecular diversity and population differentiation analyses at the broad stratification level, in order to avoid bias due to oversampling in social groups.

Within the Mariana Islands, samples were further stratified according to geography into 3-island strata (Guam, Rota and Saipan). In the Hawaiian Islands, knowledge of the social structure, habitat use, and movements (e.g. Mahaffy *et al.* 2015; Baird *et al.* 2013) was used to create three strata within the main Hawaiian Islands (MHI: Hawai‘i Island, O‘ahu/Kaua‘i Islands, and Pelagic) based on photo-identification and observation data (Fig. 1). We placed samples from the Northwest Hawaiian Islands (NWHI) in a separate stratum, as many studies have shown strong differentiation between the MHI and NWHI for other marine mammals (Martien *et al.* 2014; Courbis *et al.* 2014; Andrews *et al.* 2010). Within the Hawaiian Islands stratum, several social groups were heavily sampled in order to test for relatedness within social groups; therefore, the data set was subsampled to include no more than two individuals from each social group for all population structure analyses. All samples from the Mariana Islands stratum were used. No comparisons were conducted within the ETP due to small sample size. Nuclear and mitochondrial stratifications and sample sizes are shown in Table 1 and Figure 1.

Molecular diversity indices for all samples and for each region were calculated for both mtDNA (Theta (θ_H), haplotypic diversity (*h*), and mean nucleotide diversity (π)) and SNP genotypes (average number of alleles per locus, expected and observed heterozygosity (H_e, H_o)). Pairwise genetic differentiation was calculated among geographic regions, and among strata within regions, using F_{ST} and Φ_{ST} for mtDNA and F_{ST} for SNP genotypes. All estimates of divergence and genetic diversity were conducted using the strataG package for R.

Samples were clustered using the Bayesian program STRUCTURE 2.3.3 for SNP data. A model allowing for admixture and correlated frequencies with no population priors was used to cluster the samples into k = 1–4 groups, with ten replicates for each k. The MCMC analysis was run for 500,000 steps with a burn-in of 100,000 steps. CLUMPP (Jakobsson & Rosenberg 2007) was used to combine STRUCTURE replicates.

To test for relatedness among social groups within the Main Hawaiian Islands stratum, samples were stratified according to previously inferred social structure (Mahaffy *et al.* 2015). Relatedness was tested using a dyadic maximum likelihood estimator (Milligan 2003) in the R package Related (Pew *et al.* 2014), which implements the software program COANCESTRY (Wang 2011). Within-group relatedness was compared to the expected relatedness by permuting a random sample 1,000 times and calculating relatedness.

Results

A total of 54 nuclear SNP loci from 192 individuals (43 from Mariana Islands, 23 from ETP, 126 from Hawaiian Islands) were successfully sequenced from four enriched libraries (Fig. 2). One sample from the Mariana Islands was determined to be a duplicate of another sample in the same stratum and removed from the data set. The Hawaiian Islands stratum was subsampled to ensure unbiased analyses of molecular diversity and population differentiation. For the broadest stratification level, 50 samples were selected from the 126 sample set. For population differentiation within the Hawaiian Islands stratum, samples from heavily sampled social groups were removed so that only two samples from each social group were used in population analyses, resulting in 101 samples. Finally, a total of 40 of the 126 Hawaiian samples were obtained from stable social groups identified by Mahaffy et al. (2015), and were included in the relatedness analysis.

The mtDNA data set, including newly generated sequences and those included in Martien *et al.* (2014a) and Van Cise *et al.* (*in review*), consisted of 295 samples, including 61 samples from the Mariana Islands, 56 samples from the ETP, and 178 samples from the Hawaiian Islands (Table 1 and Fig. 1).

We detected 18 different mtDNA haplotype sequences, most of which were restricted to a single geographic region (Table 2). There was one haplotype shared between the Hawaiian Islands and the ETP and one between the Hawaiian Islands and the Mariana Islands. mtDNA diversity was much lower in the Hawaiian Islands than in the other strata, as reported by Van Cise et al. (in review; Table 3). However, SNP diversity was slightly higher in the Hawaiian Islands than the other two strata. Pairwise genetic differentiation was significant among all three regions for both mtDNA and nuclear SNPs. F_{ST} values ranged from 0.47 to 0.74 ($p < 0.001$) for mtDNA and 0.01 to 0.31 ($p < 0.001$) for nuclear SNPs (Table 4).

Within each region, pairwise genetic differentiation among strata varied widely (Table 4). In the Mariana Islands, mtDNA differentiation was significant between Saipan and both the Guam and Rota strata, but no significant SNP differentiation was found between any of the strata. In the Hawaiian Islands, mtDNA differentiation was significant between the NWHI and each of the strata in the MHI. Mitochondrial differentiation was also significant between the Hawai‘i Island stratum and the Pelagic stratum. We did not detect significant mtDNA differentiation between Hawai‘i Island and O‘ahu/Kaua‘i , which is not surprising since nearly all samples from those two strata share a single haplotype. SNP differentiation was significant between the Hawai‘i Island and O‘ahu/Kaua‘i strata, but not significant between other strata. SNP differentiation was not tested between the NWHI and the pelagic stratum due to small sample size.

STRUCTURE analysis of all samples in the adjusted data set indicated that a 2-population model had the highest likelihood, with a three population model only slightly less likely (Fig. 3). Likelihood continued to decrease as the number of populations increased (Fig. 3). In the 2-population model, samples were grouped into an ETP population and a Hawaiian/Mariana Islands population, the second population including the pelagic samples near the Hawaiian Islands (Fig. 4).

Within the Hawai‘i Island population, relatedness within social groups was tested by grouping samples according to their known social structure (Mahaffy et al. 2015). Within-group relatedness estimates for 4 social groups with more than 5 sampled individuals were all

significantly higher than expected (Fig. 5). Overall, mean relatedness within groups was higher than expected across all groups ($p < 0.001$, Fig. 5).

Discussion

Regional genetic structure

The 3 regions chosen for this study were previously shown to have significantly different mtDNA sequences (Van Cise *et al. in review*, Morin *et al.* 2015), indicating that female short-finned pilot whales do not move between these regions. Our mtDNA F_{ST} and Φ_{ST} analyses corroborate those findings using an expanded data set with additional mtDNA samples in the Mariana and Hawaiian Islands; moreover, significant SNP differentiation between each of the regions suggests that male short-finned pilot whales also exhibit little or no dispersal between these regions in the Pacific Ocean, and that these three populations are likely reproductively isolated. Nuclear (SNP) differentiation was considerably lower between the Hawaiian Islands and the Mariana Islands than between either of these island groups and the ETP, suggesting more recent gene flow between the Hawaiian Islands and Mariana Islands populations than between the ETP and either of those populations.

Two morphologically and genetically distinct types of short-finned pilot whales have been described in the Pacific Ocean (Kasuya *et al.* 1988; Oremus *et al.* 2009; Van Cise *et al. in review*). Each of the regions in this report is dominated by one of these types: the Naisa type is found in the Hawaiian Island region and the Mariana Island region, while the Shiho type is found in the ETP region. Our data indicate very little nuclear gene flow between Naisa and Shiho types in these regions. If this pattern holds throughout their range, it would merit the consideration of these two types as separate sub-species or species. Mitogenomic data indicate the existence of a third genetic clade, recently diverged from the Naisa type animals (Morin *et al.* 2015), found in several regions throughout the Pacific Ocean, including the South Pacific Islands and the Mariana Islands. This clade may be parapatric or sympatric with the Naisa type short-finned pilot whales.

The substantially lower SNP differentiation between the Hawaiian Island region and the Mariana Island region is consistent with Morin et al.'s (2015) finding that the Mariana Island region contains individuals from both the Naisa type and the third genetic clade. This region could be an area where 2 distinct, non-interbreeding types overlap, or an area where the Naisa type and the third genetic clade interbreed. SNP differentiation between the ETP and either of the island regions is much larger, due to the fact that the Shiho type is the only type found within the ETP, and the comparison between the 2 types yields much larger SNP F_{ST} values than a comparison between two island regions that share the other (Naisa) type.

Local genetic structure

High mtDNA molecular diversity within the Mariana Islands is likely due to the overlap in this region between the Naisa type and Morin *et al.*'s (2015) recently diverged third genetic clade. Mitochondrial genetic differentiation was significant between Saipan and Guam and Saipan and Rota, suggesting the possibility of separate Guam/Rota and Saipan populations, as reported by Martien *et al.* (2014a). However, we did not detect significant differentiation within the Mariana Islands using the SNP data set. These results suggest that the Saipan and Guam/Rota groups represent different populations or social groups to which there is natal

fidelity, but that there is sufficient interbreeding between the groups to homogenize their nuclear genomes. This result should be viewed with caution, however, due to the small sample size from Saipan (n = 12 in the nuclear data set) and limited number of SNPs in the data set.

Within the Hawaiian Islands, mtDNA indicated the existence of a NWHI population that is distinct from all strata in the main Hawaiian Islands. There were too few samples with SNP data (N = 2) to test for differentiation between the NWHI and MHI strata using nuclear data. Interestingly, SNP differentiation was significant between the Hawai‘i Island stratum and the O‘ahu/Kaua‘i stratum, suggesting a separate population around Hawai‘i .

Relatedness and social structure

Many social cetaceans live in social groups for their entire lives. In killer whales, these groups are known to be matrilineal and multigenerational (Ford et al. 2000; Ford et al. 2011). In long-finned pilot whales (*Globicephala melas*), relatedness was higher than expected among animals that stranded together, indicating the likelihood that this species also forms social groups of related individuals (Amos *et al.* 1993). In the Hawaiian Islands, short-finned pilot whale social groups are also highly related. It is possible that the formation of social groups among relatives may be a behavioral trait common to social cetaceans, whereas the formation of social groups comprising non-relatives is much less common (e.g. Baird & Whitehead 2000; Rendell et al. 2012).

In the Hawaiian Islands, short-finned pilot whales spend most of their time around one island or group of islands (Mahaffy *et al.* 2015); however, photo ID and satellite tagging data show that short-finned pilot whales do move among some of the islands at times (Baird *et al.* 2015). It is possible that, where physical barriers are lacking, social structure plays an important role in generating and maintaining population genetic structure among sympatric and parapatric groups. Although we know less of the social structure in the Mariana Islands, our results suggest that they may also form social groups with their close relatives, and therefore similar mechanisms could be driving population structure in that island region as well. It is possible that female natal philopatry in the Mariana Islands, as suggested by mtDNA pairwise differentiation, is driven by ties to their natal social group, thus driving divergence between islands within the Mariana Islands region. High relatedness within social groups suggests that males also remain in their natal social group. However, lack of SNP differentiation suggests male-mediated gene flow, driven by males breeding outside their island population. It is possible that males leave their natal social groups to breed, a behavior that has previously been described in killer whales (Ford et al. 2011; Pilot et al. 2010).

Future work

Analyses included in this report are preliminary. Further analysis of the data is needed to examine some of the questions presented in this report, and it is possible that some questions may only be answered with additional data, especially for strata with small sample numbers. Future work will be focused on examining the link between social structure and genetic structure in the Hawaiian Islands, as well as examining differences in population structure patterns between insular and pelagic habitats. For the latter question, it will be interesting to determine whether genetic differentiation is clinal in the ETP, using a Mantel test

to detect isolation by distance, as opposed to the insular populations found around the Hawaiian and Mariana Islands.

References

Amos, W., Schlotterer, C. & Tautz, D., 1993. Social structure of pilot whales revealed by analytical DNA profiling. *Science*, 260:670–672.

Andrews, K.R., Karczmarski, L., Au, W., et al., 2010. Rolling stones and stable homes: social structure, habitat diversity and population genetics of the Hawaiian spinner dolphin (*Stenella longirostris*). *Molecular ecology*, 19(4):732–48.

Baird, R.W., Webster, D., Aschettino, J., et al., 2013. Odontocete Cetaceans Around the Main Hawaiian Islands: Habitat Use and Relative Abundance from Small-Boat Sighting Surveys. *Aquatic Mammals*, 39(3):253–269.

Baird, R.W., Diley, A., Webster, D., et al., 2015. *Odontocete Studies on the Pacific Missile Range Facility in February 2014: Satellite-Tagging, Photo-Identification, and Passive Acoustic Monitoring*, Prepared for Commander, U.S. Pacific Fleet. Submitted to Naval Facilities Engineering Command Pacific, Pearl Harbor, Hawaii, under Contract No. N62470-10-D-3011, Task Order KB26, issued to HDR, Inc., Honolulu, Hawaii.

Baird, R.W. & Whitehead, H., 2000. Social organization of mammal-eating killer whales: group stability and dispersal patterns. *Canadian Journal of Zoology*, 78(12):2096–2105.

Barlow, J., 2006. Cetacean abundance in Hawaiian waters estimated from a summer/fall survey in 2002. *Marine Mammal Science*, 22(2):446–464.

Courbis, S., Baird, R.W., Cipriano, F., et al., 2014. Multiple Populations of Pantropical Spotted Dolphins in Hawaiian Waters. *Journal of Heredity*, 105(5):627–641.

Ford, M.J., Hanson, M.B. & Hempelmann, J.A., 2000. *Killer whales: the natural history and genealogy of Orcinus orca in British Columbia and Washington State* 2nd edn., Vancouver: University of British Colombia Press.

Ford, M.J., Hanson, M.B., Hempelmann, J., et al., 2011. Inferred Paternity and Male Reproductive Success in a Killer Whale (*Orcinus orca*) Population. *The Journal of Heredity*, 102(5):537–53.

Hamilton, T.A., Redfern, J.V., Barlow, J., et al., 2009. Atlas of cetacean sightings for Southwest Fisheries Science Center cetacean and ecosystem surveys: 1986 – 2005. NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-440. 77 pp.

Hancock-Hanser, B.L., Chivers, S., Barid, R., et al., 2013. Targeted multiplex next-generation sequencing: advances in techniques of mitochondrial and nuclear DNA sequencing for population genomics. *Molecular Ecology Resources*, 13(2):254–68.

Hodges, E., M. Rooks, Z. Xuan et al., 2009. Hybrid selection of discrete genomic intervals on custom-designed microarrays for massively parallel sequencing. Nature Protocols, 4(6), pp.960–974.

Jakobsson, M. & Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23(14):1801–1806.

Kasuya, T., Miyashita, T. & Kasamatsu, F., 1988. Segregation of two forms of short-finned pilot whales off the Pacific coast of Japan. *The Scientific Reports of the Whales Research Intitute*, 39:77–90.

Katoh, M. & Kuma, M., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30:3059–3066.

Mahaffy, S.D., Baird, R.W., McSweeney, D., et al., 2015. High site fidelity, strong associations, and long-term bonds: Short-finned pilot whales off the island of Hawai'i. *Marine Mammal Science*, 31(4): 1427-1451.

Martien, K.K., Chivers, S.J., Baird, R.W., et al., 2014a. Nuclear and Mitochondrial Patterns of Population Structure in North Pacific False Killer Whales (*Pseudorca crassidens*). *Journal of Heredity*, 105:611–626.

Martien, K.K., Hill, M.C., Van Cise, A.M., et al., 2014b. Genetic diversity and population structure in four species of cetaceans around the Mariana Islands. NOAA Technical Memorandum NMFS-SWFSC-536. 18 pp..

Meyer, M. & Kircher, M., 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harbor protocols, 2010(6), p.pdb.prot5448.

Miller, S.A., Dykes, D.D. & Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, 16(3):1215.

Milligan, B.G., 2003. Maximum-likelihood estimation of relatedness. *Genetics*, 163(3):1153–1167.

Morin, P.A., Archer, F., Pease, V., et al., 2012. An empirical comparison of SNPs and microsatellites for population structure, assignment, and demographic analyses of bowhead whale populations. *Endangered Species Research*, 19(2):1–27.

Morin, P.A., Van Cise, A.M., Martien, K.K., 2015. Mitogenome phylogeography of short-finned pilot whales in the North Pacific, with reference to the Marianas Islands. Appendix II *in* Hill M.C., A.D. Ligon, E.M. Oleson, et al., 2015. *Cetacean Monitoring in the Mariana Islands Range Complex, 2014. Prepared for the U.S. Pacific Fleet Environmental Readiness Office. PIFSC Data Report DR-15-003. 56 pp. + Appendices.*

Oremus, M., Bales, R., Dalebout, M., et al., 2009. Worldwide mitochondrial DNA diversity and phylogeography of pilot whales (*Globicephala spp*.). *Biological Journal of the Linnean Society*, 98:729–744.

Pew, J., Muir, P., Wang, J., et al., 2014. related: an R package for analyzing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources*, 15:557–561.

Pilot, M., Dahlheim, M.E. & Hoelzel, a. R., 2010. Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (*Orcinus orca*). *Journal of Evolutionary Biology*, 23(1):20–31.

R Development Core Team (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rendell, L., Mesnick, S., Dalebout, M., et al., 2012. Can Genetic Differences Explain Vocal Dialect Variation in Sperm Whales, *Physeter macrocephalus*? *Behavior genetics*, 42(2):332–343.

Sambrook, J., Fritsch, E. & Maniatis, T., 1989. *Molecular Cloning: A Labratory Manual, 2nd ed.*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Van Cise, A.M., Morin, P.A., Baird, R.W. et al., *in review*. Redrawing the map: mtDNA provides new insights into the distribution and diversity of short-finned pilot whales in the Pacific Ocean. *Submitted to Marine Mammal Science.*

Wang, J., 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular ecology resources*, 11(1)141–145.

Table 1. mtDNA and SNP sample sizes for the adjusted data sets for regional and local stratification levels.

Stratum	mtDNA samples	SNP samples
Regions		
Mariana Islands	61	42
Hawaiian Islands	178	50
ETP	56	23
Mariana Islands		
Guam	25	17
Saipan	18	15
Rota	18	10
Hawaiian Islands		
Hawai‘i	85	54
O‘ahu/Kaua‘i	57	29
Pelagic	20	16
NWHI	16	2

Table 2. Haplotype counts for each region and strata within region.

	2	4	5	6	7	8	9	10	11	12	17	18	A1	A2	C	E3	J	K
Regions																		
Mariana Islands							2				4	1	12	39	3			
Hawaiian Islands	1								1	5					1		168	2
ETP	6	1	1	3	1	2		1								41		
Mariana Islands																		
Guam											2			20	3			
Rota							2						3	13				
Saipan											2	1	9	6				
Hawaiian Islands																		
MHI	1								1						1		157	2
NWHI										5							11	

Table 3. Molecular diversity indices for mtDNA and SNP data sets.

	mtDNA ***N***	**θ_H**	**Haplotype diversity (*h*)**	**Nucleotide diversity (π)**	**SNP** ***N***	**Ave. num alleles**	**H_o**	**H_e**
All samples	295	0.475	0.638	0.003	115	2.9	0.42	0.47
Regions								
Mariana Islands	61	0.412	0.554	0.001	42	3.04	0.38	0.42
Hawaiian Islands	178	0.081	0.109	0.0003	50	2.66	0.43	0.44
ETP	56	0.339	0.455	0.0008	23	3.02	0.3	0.33

Table 4. Pairwise genetic differentiation (θ_{ST} and F_{ST}) among and within regions for mtDNA and SNP data.

Stratum	mtDNA N	mtDNA F_{ST}	p	mtDNA θ_{ST}	p	SNP N	SNP F_{ST}	p
Among Regions								
Mariana Islands vs. ETP	61/56	0.49	0.001	0.46	0.0001	42/23	0.31	0.001
Mariana Islands vs. Hawaiian Islands	61/178	0.75	0.001	0.85	0.0001	42/50	0.01	0.001
Hawaiian Island vs. ETP	178/56	0.79	0.001	0.91	0.0001	50/23	0.29	0.001
Within Mariana Islands								
Guam vs. Rota	25/18	0.03	0.13	0.04	0.12	17/15	0.01	0.09
Guam vs. Saipan	25/18	0.31	0.001	0.19	0.003	17/10	0.01	0.32
Rota vs. Saipan	18/18	0.17	0.012	0.17	0.006	15/10	-0.01	0.79
Within Hawaiian Islands								
Hawai‘i vs. O‘ahu/Kaua‘i	85/57	0.02	0.14	0.01	0.14	54/29	0.01	0.05
Hawai‘i vs. Pelagic	85/20	0.2	0.008	0.18	0.007	54/16	0.01	0.15
Hawai‘i vs. NWHI	85/16	0.58	0.001	0.58	0.001	54/2	0.01	0.31
O‘ahu/Kaua‘i vs. Pelagic	57/20	0.04	0.10	0.04	0.099	29/16	-0.001	0.51
O‘ahu/Kaua‘i vs. NWHI	57/16	0.35	0.001	0.26	0.001	29/2	0.03	0.14
Pelagic vs. NWHI	20/16	0.11	0.04	0.06	0.11	16/2	NA	NA

Figure 1. Sample locations for SNP only (black), mtDNA only (red) and SNP/mtDNA samples (blue) used in the study. Black circles indicate group stratification for pairwise differentiation analyses. Top: Regional stratification. Bottom: local stratification within each region.

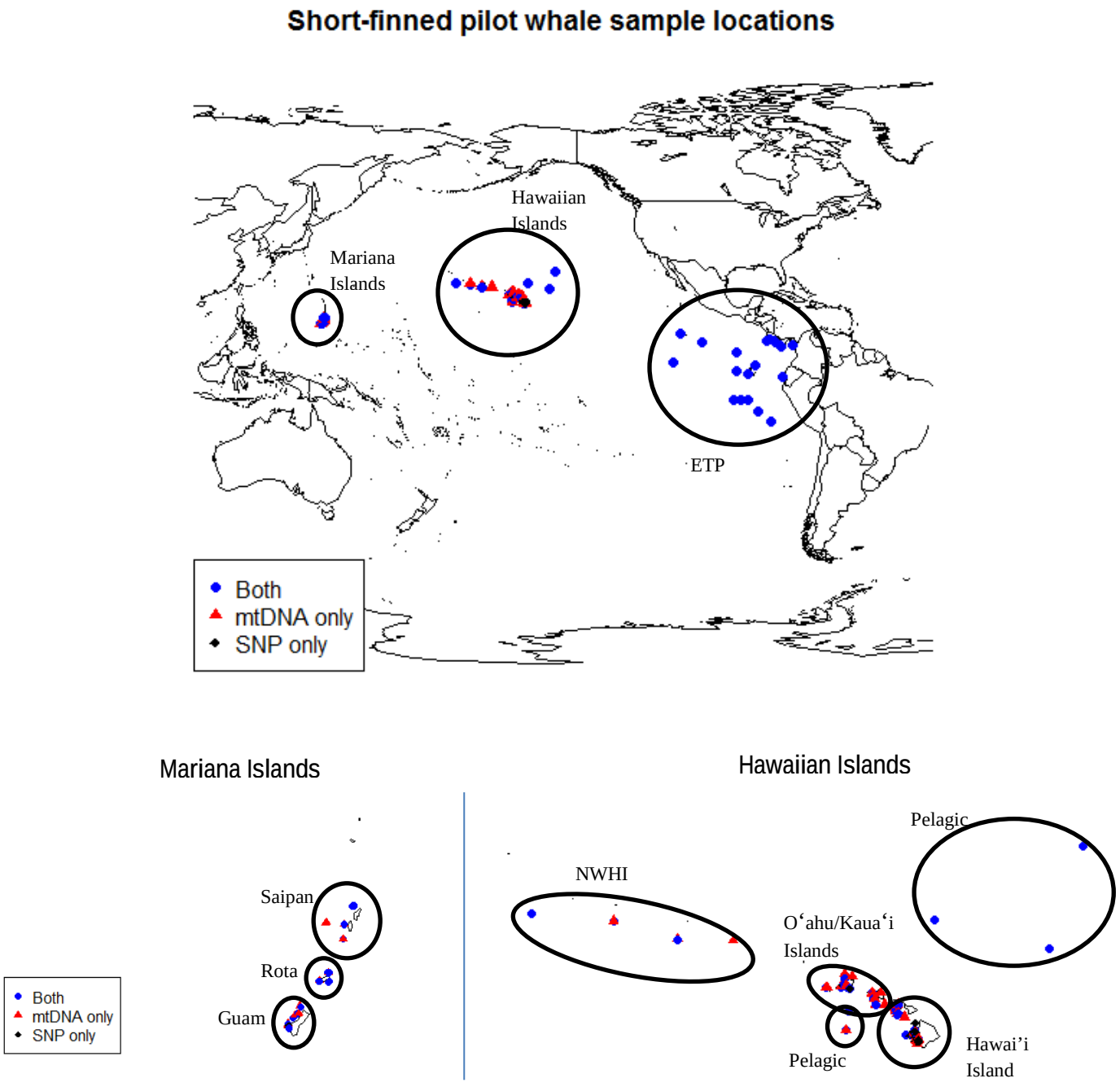


Figure 2. Histogram of the %SNPs genotyped for each sample included in this report, showing some SNP loci with low genotype success rates.

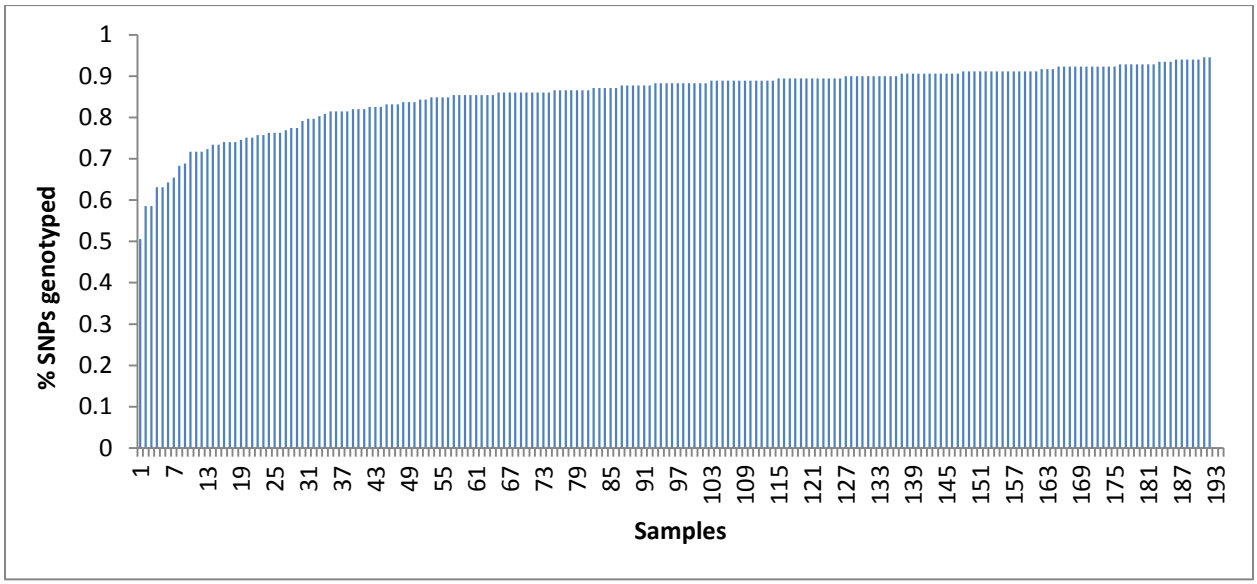


Figure 3. Posterior probability and Evanno likelihood metrics from STRUCTURE analysis of SNP data, indicating strong likelihood of two regional populations (K).

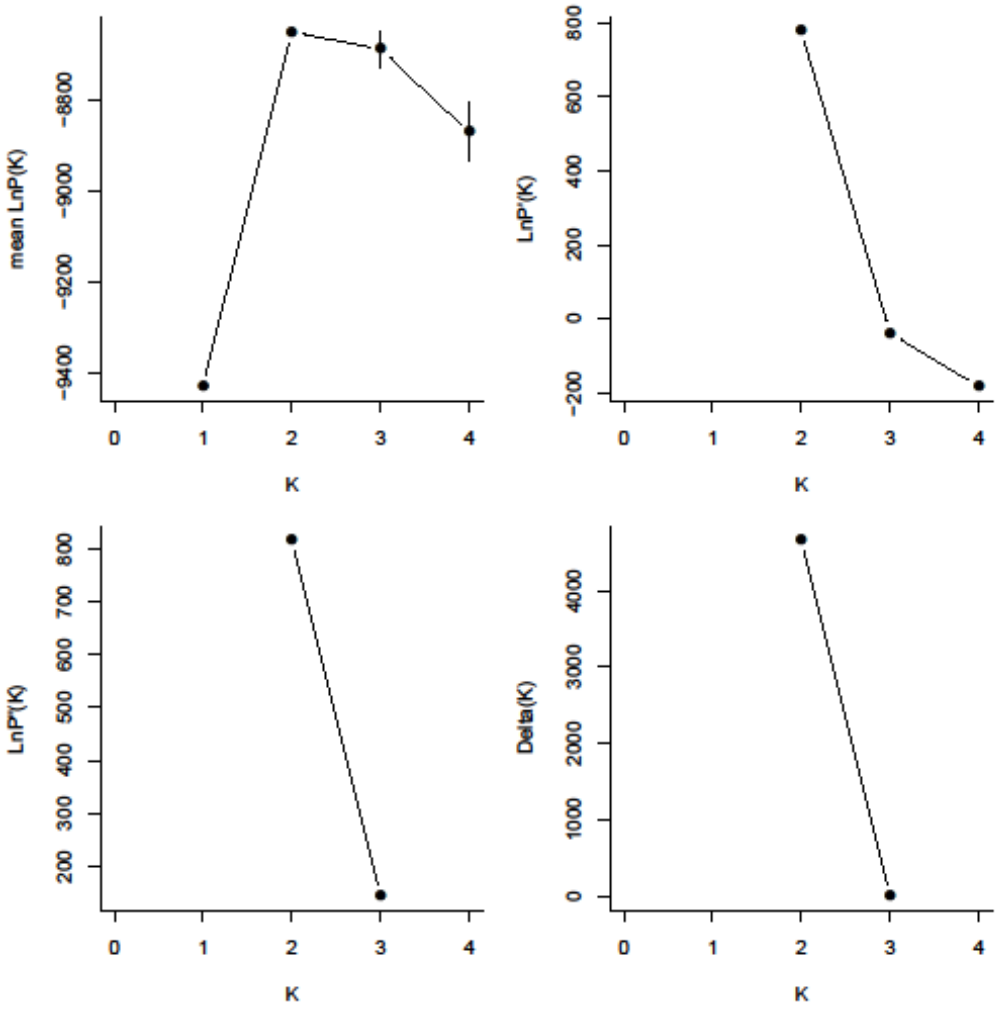
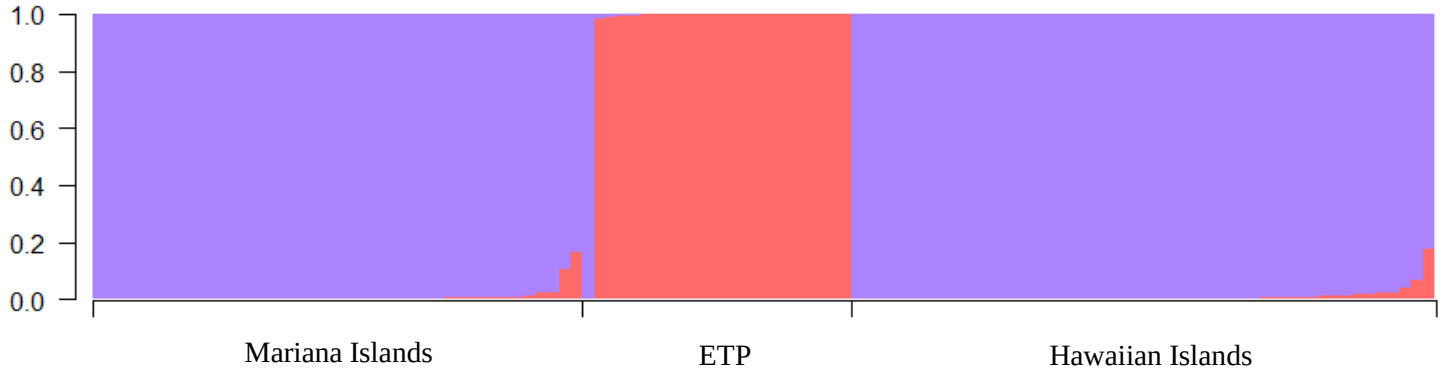


Figure 4. Stratification of samples into a) two or b) three populations based on STRUCTURE analysis. Samples are sorted by assignment probability. Additional genetic structure was not detected at higher population numbers.

a)



b)

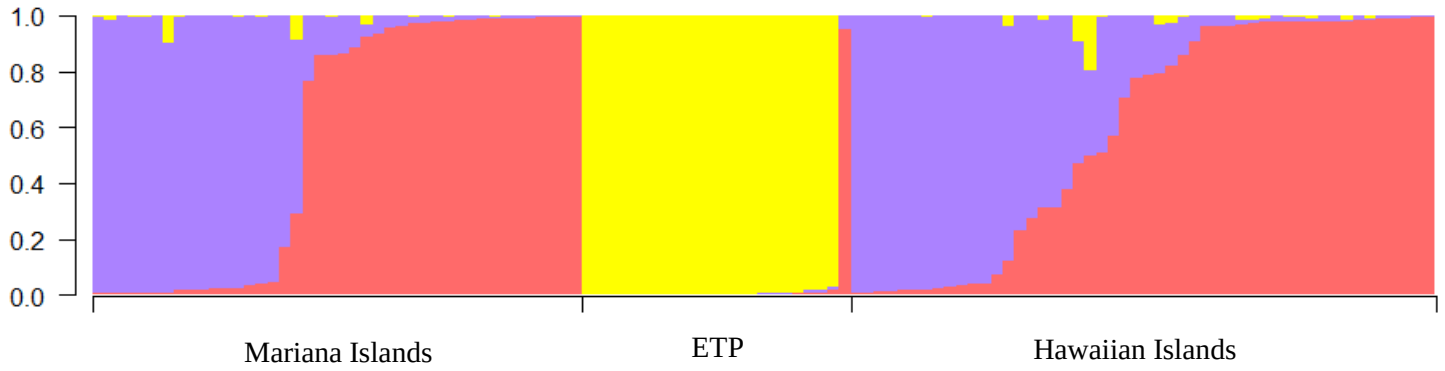


Figure 5. Within-group relatedness (red arrows) compared with expected relatedness values from a randomly selection of the whole sample. B1, E, G and H are social groups identified by Mahaffy et al. (2015). Final panel indicates overall group relatedness, where the observed average relatedness within groups is compared to expected values across all groups.